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STREAMING AND POLARITY IN *MASTIGINA HYLÆ* (FRENZEL).

ELERY R. BECKER,

IOWA STATE COLLEGE.[1]

Mastigina hylæ, a member of the order Rhizomastigina, and an inhabitant of the rectum of the bullfrog tadpole, is from a strictly morphological standpoint a flagellate. The nucleus lies in the extreme anterior end, and near it are to be found the basal body which gives rise to a single, inconspicuous flagellum, and certain intracytoplasmic structures which the writer (1925) has previously described. From a physiological standpoint, however, the organism is an amœba, for the cytoplasm is continuously engaged in a process of "fountain streaming," and the single flagellum is inactive so far as can be observed. Before this flagellum was noted, the writer (1923) published a note in which he referred to this organism as "an entozoic amœba." This miscomprehension of the structure of the cell led to certain misinterpretations of the experiments which were reported in that note.

It is convenient to divide the substance in a streaming *Mastigina hylæ* into three zones: (1) the anterior zone, consisting of the nucleus, basal granule and associated structures, and the gelled cytoplastic matrix in which these structures are embedded (Fig. 1*a*); (2) a semi-fluid posterior or tail region, whose substance takes no part in the general cytoplasmic streaming (Fig. 1*c*); (3) the more extensive intermediate streaming region (Fig. 1*b*). The cell substance and ingested materials of this intermediate zone are reflected posteriad from the posterior surface of the nucleus and remaining portions of the anterior zone and anteriad from the concave anterior surface of the tail region.

[1] The experiments reported in this paper were begun while the writer was located at Princeton, and completed at Iowa State College during the summer of 1926.

It is then the intermediate zone which is almost entirely involved in the process of streaming. We may, like Prof. John A. Ryder (1893), conceive of this streaming system as having the configuration of an elongated smoke ring, a system in which "some of itself may continuously flow through itself in order to make the progressive forward movement of itself possible." There is an axial stream which flows swiftly and continuously toward the anterior end (Fig. 1). Upon encountering the posterior surface of the nucleus and nearby region the materials in this axial stream are deflected radially to the periphery and then reflected backwards about the axial stream, forming a hollow cylinder of "ectoplasm" about it whose contents flow counter to it. The cylinder substance enters the axial stream again in the posterior region, although some part of it may have done this before the posterior region is reached. Actual timing shows that it takes from 8 1/2 to 11 5/6 seconds for a particle to make the complete circuit in healthy streaming cells. It is important to keep in mind that the nucleus is constantly at the anterior end of the long axis of the cell, where it may appear to oscillate slightly from side to side.

At any instant the living *Mastigina* has somewhat the shape of a helix of from one and one fourth to two complete coils. The coils of the helix are being constantly formed at the anterior end, while they fade out at the posterior end. The shape of the body is due to the fact that the nucleus and surrounding medium is jostled about somewhat after the fashion of the ball in the vertical jet of water which is sometimes seen in a shooting gallery. This accounts for the apparent oscillation from side to side as observed under the low power of the microscope. As a consequence the materials in the axial stream are deflected radially somewhat ununiformly, and the body is curved toward the arc of the cross-section receiving the lesser amount at any instant. Since the streaming is uninterrupted and steady, the natural resultant of the various factors is the construction of a helix. Sometimes, however, the organism is almost spatulate in shape.

The progressive movement of the organism as a whole is not usually the maximum to be expected for it often remains attached by its tail. It was found by actual timing that the maximum forward locomotion actually obtainable was about 480 microns per

minute. The rate may be reduced to approximately zero, depending on how much the attachment of the uroid impedes progress.

The application of the colloid theory of protoplasm to amœboid movement is now so well recognized that it is hardly necessary to point out that the axial stream is the plasmasol (See Mast, '23, '26), the protoplasm about is the plasmagel. A thin tough elastic plasmalemma encloses the entire protoplasmic substance. If this is suddently broken, as by an abrupt jab with a glass needle, the entire protoplasmic mass except the nucleus and gelled anterior end goes into solution in the water medium. Flakes of the plasmalemma can also be seen floating about.

Here is a cell with a definite polarity. Should this polarity be ascribed to some factor inherent within the general cytoplasm? Or are the direction of streaming and consequently the cell axis maintained by virtue of some property of the nucleus, whether physical, chemical, or physico-chemical, which determines that the direction of the axial stream shall always be toward the nucleus? Or does the differential factor which determines the direction of the streaming operate in the anterior zone independently of the nucleus?

A number of individuals were cut squarely either across the middle or slightly anterior to the middle by means of a finely drawn hard-glass needle operated in a Chamber's micromanipulator. In every case the anterior fragment is able to resume the normal shape and streaming almost instantly (Fig. 3). The posterior fragment always rounds up somewhat, undergoes some fitful and uncoördinated cyclosis, extends and retracts a number of pseudopods from its cortical region, all without accomplishing any appreciable change of position (Figs. 2 and 4). The ability to carry on the gelation-solation process (the ectoplasmic-endoplasmic proces) does not exist within it. In about eight minutes it rounds up and streaming ceases completely. The anterior region continues to behave quite normally, even though it be as small as one fifth the area of the entire organism. It is not even necessary to sever the two areas completely, for the application of pressure by a needle held transversely to the large axis at any point in the intermediate zone will bring about the same result (Fig. 6).

When the pressure is released the two areas flow together and streaming is resumed.

If only the nucleus and a negligible amount of cytoplasm be amputated, the result as regards the enucleated amœba is the same. These observations decide definitely against the view that the direction of movement is determined by some factor inherent in the ultramicroscopic structure of the cytoplasm. Thus the differential factor which determines the direction of streaming is either in the nucleus or in the gelled cytoplasm at the extreme anterior tip. It cannot be held that streaming was arrested simply by removal of nuclear influence, for the writer (1926) and others have shown that cessation of streaming is not an immediate effect of the removal of the nucleus in amœbæ.

Oftentimes in unsuccessful attempts to cut off the entire anterior end (Fig. 7), the pressure applied served to dislocate the nucleus (and all the other anterior structures with it as was subsequently proved), and it commenced to travel toward the posterior end in the outer gel. A new anterior tip was temporarily established (Fig. 8). But always after a moment the streaming was arrested, a bulge appeared at the point where the nucleus had stopped, endoplasm flowed toward the nucleus, and the organism traveled away at right angles to its former course (Fig. 9). The uroid or tail piece seems also to be a permanent structure, for it again resumes its position at the posterior tip, and is not formed anew. Centrifuged specimens likewise show similar temporary disturbances of polarity, but these are more difficult to interpret. Experiments of this nature lend further support to the view that the factor that determines the direction of streaming is located somewhere in the anterior tip, for it persists in resuming its natural position at the anterior pole of the cell even though the polarity be temporarily disturbed.

But does this differential factor lie in the nucleus as the writer (1923) formerly supposed? In the summer of 1926 while attempting to cut off the anterior tip of one of these organisms by the free-hand method the nucleus was accidentally squeezed from the vacuole which it normally occupied, while the remaining anterior structures were undisturbed. The vacuole which the nucleus formerly occupied was plainly visible as a less refractile space in

the anterior end. The nucleus traveled with the food particles, making time after time the circuit from axial stream to anterior end, backwards in the outer layer of gelled protoplasm to the posterior end, and then again into the axial stream. The polarity of the cell was not in the least disturbed. This makes necessary a reversal of the former conclusion of the writer that "the nucleus seems in some way to play a rôle in directing endoplasmic streaming, presumably by its ability to slightly liquefy the ectoplasmic gel, lowering the resistance at certain points to the force of the endoplasmic stream." This conclusion, as stated above, was based upon a misconception of the structure of the anterior end of the organism.

The more probable status of affairs is that it is the entire anterior tip of permanently gelled protoplasm which prevents gelation of the "endoplasm" behind it, and which by imperfect continuity with the outer gelled layer of the protoplasm of the intermediate zone creates a circular zone of weakened elasticity or lowered resistance to the internal pressure of the plasmasol When the anterior tip is amputated the remainder of the organism tends to round up. In such cases the plasmasol is imprisoned within a wall of plasmagel for the penetration of which it cannot muster enough internal pressure, thus making impossible the solation-gelatin process and the resultant streaming. If a normally streaming individual be agitated mechanically, it too rounds up, just as an enucleated fragment. This is known as the stimulated condition. But if left quietly for a moment one or several pseudopods are thrust out, but it is always the one with the nucleus in its distal tip which becomes the permanent anterior end of the streaming organism. The possession of the anterior zone is thus the *sine qua non* for normal streaming in this protozoön.

The writer has in rare instances observed in fragments of *Amœba dubia* which he had enucleated streaming strikingly similar to that in *Mastigina hylæ*. In such cases the contractile vacuole must be in the enucleated fragment and lie at the edge. It sometimes comes to occupy a position similar to that of the nucleus in *Mastigina hylæ* (Fig. 5). This type of streaming commences an instant after enucleation and may continue for from one to two minutes before the contractile vacuole bursts. The streaming is

usually unbelievably rapid while it lasts. Such instances would certainly justify the assumption that polarity is determined by the physical presence in the gel of a foreign body which lowers the resistance of the peripheral zone to the internal pressure in its immediate vicinity.

Conclusion.

Mastigina hylæ shows typical fountain streaming, and during locomotion assumes the shape of a helix with the nucleus at the anterior end. If the organism be cut into an anterior and a posterior fragment, the anterior fragment continues to stream normally, while the posterior fragment rounds up, shows uncoördinated streaming and pseudopod formation, and soon dies. If just the extreme anterior extremity be severed from the cell, the entire protoplasmic mass behaves just as the above mentioned posterior fragment. This indicates that the factor which determines the direction of streaming, and hence polarity, is located in the anterior end. That the nucleus is not immediately responsible for polarity is indicated by the fact that polarity is not disturbed if it is dislodged from the vacuole it occupies without disturbing the remaining anterior structures. If the entire anterior end is made to travel posteriad with the outer gel, and a new anterior end formed, the original polarity is resumed by a cessation of streaming, and renewed streaming toward the nucleus.

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DESCRIPTION OF PLATE.

FIG. 1. A normally streaming *Mastigina hylæ* showing the three zones into which the body may be divided. Arrows show direction of streaming.

FIGS. 2 AND 3 show diagrammatically the difference in the behavior of fragments from the posterior and anterior portions of the body respectively. The latter streams normally.

FIG. 4. An enucleated fragment sending out broad pseudopods from the cortical gel (ectoplasm). The sol (or endoplasm) is confined closely within the gel.

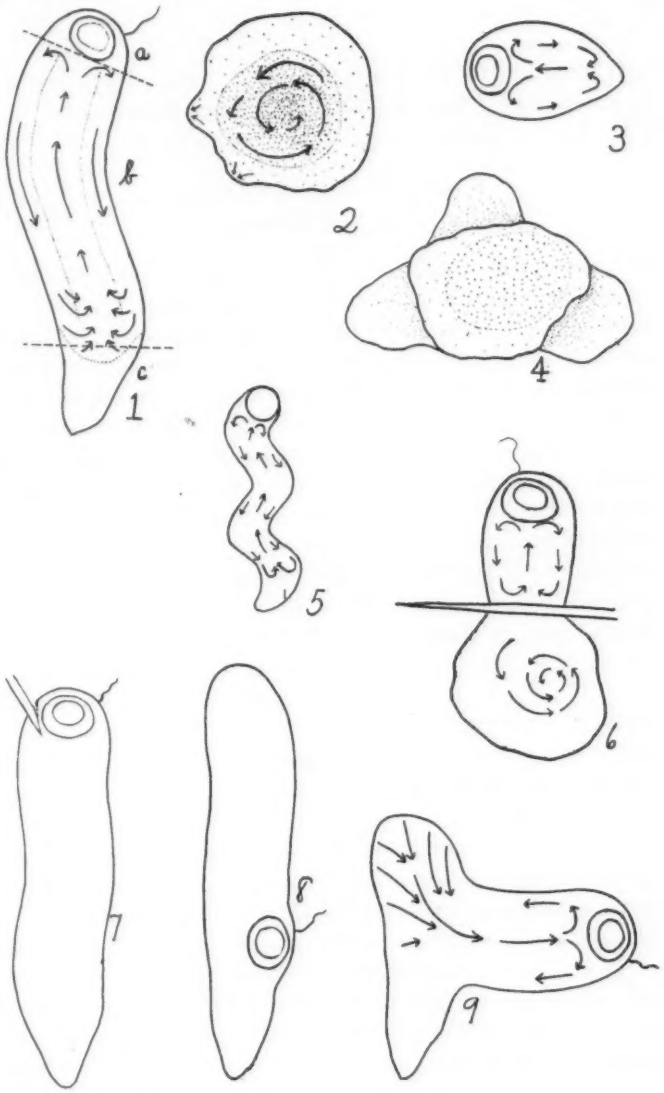
FIG. 5. An enucleated fragment of *Amœba dubia* streaming after the fashion of *Mastigina hylæ* with a contractile vacuole in the anterior tip.

FIG. 6. The effect of pressure at right angles to the longitudinal axis upon the streaming of *Mastigina hylæ*.

FIG. 7. Illustrating the method by which a break was made in the outer gel. This permitted the substance of the axial stream to rush out.

FIG. 8. The nucleus, etc., has passed to the posterior end, and a new anterior end temporarily formed.

FIG. 9. The reversal of streaming, in which the nucleus resumes its former position and the former polarity is restored.



ELERY R. BECKER.

STUDIES ON THE LIFE CYCLES OF TWO SPECIES OF FRESH-WATER MUSSELS BELONGING TO THE GENUS *ANODONTA*.[1]

MARY ELIZABETH TUCKER,

UNIVERSITY OF ILLINOIS.

Following the pioneer work of Lefevre and Curtis (1911) in this country, much interest has centered around the investigation of the developmental cycles of the fresh-water mussels. Numerous studies have demonstrated that all members of the family Unionidæ undergo sexual development through a larval stage known as the glochidium. While Surber (1912) and others have called attention to the differences in the glochidia of many species, there are as yet many untouched possibilities of differentiating easily confused species and varieties on the basis of glochidial characters. Until quite recently it has been held that in all but two of the numerous species within this family, the glochidium must pass through a period of parasitic existence on an aquatic vertebrate before transformation to the juvenile mussel is possible. With few exceptions, fishes serve as the hosts essential for the parasitic glochidia.

It was in 1866 that Leydig made the discovery that the glochidium, after leaving the parent, completed its development as a parasite on a fish. Following that disclosure, it was believed that the parasitic life was a necessary phase in the life cycle of any fresh-water mussel. Lefevre and Curtis (1911) observed a species, *Strophitus edentulus*, which undergoes metamorphosis without parasitism, the first case cited of such a condition among the Unionidæ. This report marked an important advance in the history of the knowledge of these forms.

Three years after the discovery by Lefevre and Curtis that *Strophitus edentulus* passes through its metamorphosis in the entire absence of parasitism, Howard (1914) reported a second

[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 305.

case of such development. He declared (1914: 353): "One of the mussels for which I found no natural infection and for which none have been reported was *Anodonta imbecillis* (Say)." Howard stated further (p. 354): "I have tested the reaction of the glochidia in the presence of fish and obtained evidence that they do not respond as other known parasitic forms."

As a result of further investigations, Howard (1911: 355) presented the account of obtaining both infection and encystment of *Anodonta imbecillis* glochidia. He also reported having obtained infection and encystment of *Strophitus edentulus*, and for this species in addition, declared actual metamorphosis to occur on fish. He concluded, therefore, that *Strophitus edentulus* glochidia may be facultatively parasitic, while for *Anodonta imbecillis*, "there is at least a persistence of the parasitic reaction when the glochidia are artificially brought in contact with fish."

As a result of investigations carried on at the University of Illinois, some observations have been made on the life histories of two species of fresh-water mussels belonging to the genus Anodonta, *Anodonta imbecillis*[1] and *Anodonta grandis*. Attention has been directed especially to a study of the glochidia and to their metamorphoses. In this connection, the occurrence of metamorphosis without parasitism has been restudied in *Anodonta imbecillis* and observations have also been made of both glochidia and juvenile mussels of *Anodonta imbecillis* and *Anodonta grandis*.

This work has been carried on under the supervision of Professor H. J. Van Cleave at the University of Illinois. The writer wishes to express to Professor Van Cleave appreciation for suggestions and guidance in this study. Further indebtedness is hereby acknowledged to R. E. Richardson for the identification of fishes used, and to F. C. Baker for aid in ascertaining the species of mussels concerned.

[1] Baker (1927*b*) created a new genus, *Utterbackia*, with *A. imbecillis* as type. Evidences presented in this paper eliminate one of the principle characters for differentiating this genus from *Anodonta*. Under these circumstances it seems desirable to use the older name in this publication until the exact status of *Utterbackia* is determined.

Metamorphosis without Parasitism.

Development of Strophitus edentulus.—When Lefevre and Curtis (1911) recorded their observations on the direct development of *Strophitus edentulus*, they stated that the young mussels had developed inside cords within the marsupia, reaching the stage "attained by any other unionid at the time of liberation from the host fish." The young at this stage were liberated from the marsupia by the disintegration of the cords and showed "characteristic features resembling those of *Anodonta* at the close of the parasitic period." The authors reported that they were unable to bring about the attachment of these young to fish. Since the juveniles crept about on the bottom of the dish after the manner of the young of other species in post-parasitic life, Lefevre and Curtis concluded that no subsequent parasitism was possible.

Case of Anodonta imbecillis.—Howard's discovery of *Anodonta imbecillis* passing through its metamorphosis in the absence of parasitism, brought to light a second case of this newly disclosed means of development. He secured several specimens of *Anodonta imbecillis* the latter part of November (1913). Since the species is hermaphroditic (Sterki 1898: 87), his expectations were confirmed in that all the specimens were gravid. The marsupial contents upon examination showed what at first appeared to be glochidia to be in reality "juvenile mussels with organs developed to the stage usually seen at the end of parasitism when the young mussel escapes from its host" (1914: 353–4).

In confirmation of his conclusion as to the development of *Anodonta imbecillis*, Howard stated (1914: 354): "I have tested the reaction of the glochidia in the presence of fish and obtained strong evidence that they do not respond as other known parasitic forms." He does not record, however, the species of fish used. Mature glochidia taken in March were employed which "in an exposure to fish for an hour failed to give the usual infection. A few glochidia lodged in the mouths of the fish, but no encystment could be detected. The fish showed no response." He placed especial significance upon the fact that the glochidia of *Symphonota complanata* rapidly became attached to the fish which showed considerable uneasiness in marked contrast to the behavior of fishes in the presence of *Anodonta imbecillis* glochidia.

Upon this premise, Howard thought that he had conclusive evidence that *Anodonta imbecillis* passes through its metamorphosis in the absence of parasitism.

As a result of further investigation, Howard was forced to modify his conclusions, for he reported (1914: 355) both infection by and encystment of *Anodonta imbecillis* glochidia. Howard does not record the host used in his experiments, but, since the susceptibility of fishes is significant in this connection, it is possible that in his earlier experiments he used a species entirely immune to *Anodonta imbecillis*.

Occurrence of Parasitism in *Anodonta imbecillis*.

In the present investigations, it has been found that *Anodonta imbecillis* not only discharges glochidia but that they are capable of attachment upon a fish (*Apomotis cyanellus*). The period of parasitic life is of practically the same duration as is necessary for the metamorphosis of *Anodonta grandis*.

In the course of this investigation, constant reference has been made to a comparison between conditions in *Anodonta imbecillis* and *Anodonta grandis* for the developmental cycles of these two species has been studied from a comparative point of view.

Three individuals of *Anodonta imbecillis* obtained from Lake Decatur at Decatur, Illinois, in November, 1926, were placed in a tank of running water and kept under observation. Of these, one died December 3 and was found to contain only a few living glochidia in the outer gills. The remaining two specimene wers placed in separate containers January 27. On February 1, one of these was found to have given off living glochidia which were recovered by a pipette from the bottom of the jar. During a period of twenty-four hours following, approximately 200 glochidia were discharged, which were followed by similar numbers until February 8, when the mussel was found with gaping valves. Upon examination the marsupia showed a very few of the larvæ. In the meantime, the second specimen of *Anodonta imbecillis* (2/4/27) also gave off living glochidia. After two weeks, these ceased to be discharged, the mussel living, however, until March 17, at which time an examination of the marsupia showed a

few glochidial shells present but no living glochidia. It is possible that these glochidia were given off unnaturally as a result of changed environmental conditions. At any rate, the discharge was unquestionably of mature glochidia and not of young juvenile mussels.

A fairly large proportion of the glochidia discharged were active, opening and closing their valves in characteristic fashion, but in so far as these observations went were incapable of locomotion. A small green sunfish (*Apomotis cyanellus*) was subjected to a suspension of some 450 of the glochidia which had been discharged by the second specimen of *Anodonta imbecillis*. A count made after thirty minutes of exposure showed more than fifty of the larvæ attached in fairly equal distribution to the several fins of the fish. After twenty-one hours, the attached glochidia were surrounded by host tissue, encystment having been complete for at least part of the larvæ.

The infected fish was observed almost daily for the numbers of glochidia remaining. During the first few days a considerable dropping off of originally attached larvæ was noticeable. It is probable that individuals having obtained poor attachment were thus early lost.

On the eighteenth day after infection, two juveniles were recovered from the bottom of the battery jar containing the host fish. On the following days additional juveniles were secured. The following table records the number of attached larvæ and the duration of parasitism.

TABLE I.

RECORD OF THE NUMBERS OF INFECTIONS AND THE DURATION OF PARASITISM IN *Anodonta imbecillis*.

Apomotis cyanellus.	Feb.					March.									Duration of Infection.
	19	21	23	25	28	2	4	5	7	8	9	10	11	12	15	
Glochidia attached.	50	50	40	35	30	25	23	22	22	19	12	6	2	1	0	18–22 days
Juveniles recovered										2	4	3	1	1		

While Howard has reported *Anodonta imbecillis* to undergo metamorphosis without parasitism, the results of the present

study do not confirm his results. This divergence in results indicates the possibility of recognizing two distinct physiological varieties of *Anodonta imbecillis*. Howard does not give a description of the species, but the characters of the glochidia presented by Surber for *Anodonta imbecillis* agree in detail with those of the species here studied. Since preserved specimens which were collected at various times of the year yielded only glochidia, showing no evidences of transformation to the juvenile stage, it is necessary to conclude that the young of *Anodonta imbecillis* forming the basis of this study are discharged as glochidia. According to the present observations, the glochidia were given off under the conditions already stated, and some actually underwent metamorphosis upon the green sunfish (*Apomotis cyanellus*).

In this connection, it may be worth mentioning that Howard presumably worked with specimens from a large stream (the Mississippi River), while the individuals forming the basis of this study were all from relatively small streams and an artificial lake formed by damming a small stream. Utterback (1916: 2) and Baker (1927a: 112) have shown that stream conditions profoundly influence the features of the adult shell in Unionidæ. Later work supplementing that given in this paper may lead to the recognition of correlation between the differences in breeding habits and environmental factors.

The Life Cycle of *Anodonta grandis*.

As mentioned earlier in this paper the observations and experiments on the development of *Anodonta imbecillis* have been paralleled by a series of similar studies on another species, *Anodonta grandis*.

A number of living specimens of *Anodonta grandis* were obtained from Lake Decatur, Decatur, Illinois, in November, 1926. A few were examined at once and found to be gravid. The marsupia were greatly distended bearing enormous numbers of living glochidia. Measurements of the shells were made from living glochidia supplemented later by measurements from permanent microscopic mounts. Table II. records the measurements taken.

TABLE II.

RECORD OF MEASUREMENTS OF GLOCHIDIA OF *Anodonta grandis*.

	Place and Date of Collection.	Extremes of Measurement.		Most Frequent Size.	
		Length.	Depth.	Length.	Depth.
Specimen No. I...	Decatur, Ill., 11/6/26	0.350 mm.–0.390 mm.	0.343 mm.–0.382 mm.	0.358 mm.	0.350 mm.
Specimen No. II..	Decatur, Ill., 11/6/26	0.350 mm.–0.398 mm.	0.358 mm.–0.390 mm.	0.366 mm.	0.358 mm.

In the literature, there is a marked discrepancy between the measurements recorded for the glochidia of *Anodonta grandis*. Thus Surber (1912: 8) states that specimens which he studied were 0.41 mm. by 0.42 mm., while Ortmann (1919: 139) found the glochidial shells to be 0.36 by 0.37 mm. The latter author called attention to the difference between his measurements and those of Surber but offered no explanation for the disagreement.

Conchologists have long recognized a subspecies of *Anodonta grandis* characteristic of the large rivers under the name of *Anodonta grandis gigantea*. The differentiation of this variety from the typical *Anodonta grandis* has been largely upon shell characters. It is known that the material examined by Ortmann (1919: 140) was from a small stream as was also the material upon which the present investigation was based. On the other hand, it is probable that Surber's material, though recorded as *Anodonta grandis*, was from the Mississippi River and consequently belonged to the variety *Anodonta grandis gigantea*. Thus it seems reasonably certain that *Anodonta grandis* and its variety *Anodonta grandis gigantea* may be differentiated with greater certainty on the basis of glochidial measurements than on shell characters of the adult.

Under conditions of the experimentation, specimens of *Anodonta grandis* kept in tanks of running water continued to retain living glochidia within the marsupia from November 22 until January 6. Following this date, some of the females bore glochidia in the anterior region of the marsupia only, while after February 10 all the females examined lacked living glochidia. According to observations of Coker, Shira, Clark, and

Howard (1921: 142), females of *Anodonta grandis* bearing glochidia have been observed in December, January, February, and March only. Thus while there is a slight difference between the observations recorded by these authors and those of the present study, the length of the gravid period is almost identical in the two instances.

Four days after the living specimens of *Anodonta grandis* had been placed in the tank, material examined from the bottom contained living glochidia. In two weeks the number of living ones was greater in a similar quantity of material, which contained also many glochidial shells. The dates of gravidity for those specimens kept under observation in running water have already been indicated. Three living specimens brought in from the field on May 7, 1927, contained no glochidia.

In order to observe the results of artificial infection with *Anodonta grandis* for a comparison with the earlier study on *Anodonta imbecillis*, several specimens of small fishes were used. One species of minnow (*Pimephales notatus*), two of darters (*Etheostoma cæruleus*, *Beleostoma nigrum*), one sucker (*Moxostoma breviceps?*), and the green sunfish (*Apomotis cyanellus*) were placed in the tank with the living *Anodonta grandis* December 6, 1926. At this time, a fairly large number of living glochidia were present in the material at the bottom of the tank. A record was kept of the numbers of glochidial attachments for three weeks. The results are shown in the following table.

TABLE III.

RECORD OF THE ARTIFICIAL INFESTATIONS BY GLOCHIDIA OF *Anodonta grandis*.

Fish Introduced into the Tank Dec. 6, 1926.	Numbers of Infestations.					
	Dec. 8.	Dec. 13.	Dec. 16.	Dec. 18.	Dec. 23.	Jan. 3.
Pimphales notatus:						
Specimen No. I.	1	1	3	2	1	5
Specimen No. II.	0	1	3	2	1	2
Specimen No. III.	0	3	1	1	1	0
Etheostoma cæruleus	0	2	14	17	11	0
Beleostoma nigrum	0	1	4	5	2	0
Moxostoma breviceps?						
Specimen No. I.	0	0	21	37	5	2
Specimen No. II.	0	0	2	2	0	1
Apomotis cyanellus	1	1	1	4	5	5

The results in the case of the minnows, darters, and sucker showed considerable variation in numbers of infestations. Increased attachments were evident in all cases after the first infection, which condition was especially marked in the case of one darter (*Etheostoma cæruleus*) and one sucker (*Moxostoma breviceps?*). Particularly in the case of the darter with its habit of remaining near the bottom of the tank darting about actively at times, it is not surprising to find that fairly heavy infection resulted. In the cases of the fish first mentioned, the increased infections were shortly followed by a dropping off of the glochidia, as indicated by the reductions of the numbers carried. Examination of the fish showed parts of the fins sloughed off presenting noticeably ragged appearances especially marked in the case of the darters and suckers. The sunfish, on the contrary, did not show such a reduction in the numbers of glochidia. The fins remained intact, and examination soon showed the glochidia encysted. The sunfish was the only host encountered in these experiments which retained the parasites for any appreciable length of time. Lefevre and Curtis (1910: 103) recorded several species of Centrarchidæ as natural hosts of *Anodonta grandis*.

In order to observe the results of attachment of the glochidia to the sunfish more accurately than was possible by leaving the fish free to swim about in the tank, two small uninfected specimens were used for experimentation. Of these, one was a small green sunfish (*Apomotis cyanellus*), the same species which had appeared earlier to be subject to infection, and the other a rock bass (*Ambloplites rupestris*). A fairly large quantity of living glochidia from one of the gravid females of *Anodonta grandis* was placed with each of these fish in separate small containers. Infection was evident at once, and after fifteen minutes of exposure, each fish was placed in fresh water, again in the small containers which were now set in the tank in order to keep the water at a temperature favorable for the existence on the fish, at the same time preventing additional infection.

The two fishes kept under observation were kept in the same temperature conditions, the glochidia which they carried undergoing metamorphoses in eighteen days and in seventeen to twenty days respectively. A record of the duration of parasitism on the different fishes is given in Table IV.

TABLE IV.

RECORD OF THE NUMBERS OF INFECTIONS AND DURATION OF PARASITISM OF *Anodonta grandis*.

	Feb.							March.					Duration of Infection.
	16	18	19	21	23	25	28	2	4	5	7	8	
Apomotis cyanellus:													
Glochidia attached.....	2	2	2	2	2	2	2	2	2	0			18 days
Juveniles recovered.....										2			
Ambloplites rupestris:													
Glochidia attached.....	40	40	35	30	25	20	17	6	4	1	1	0	17–20 days
Juveniles recovered.....									2		3		

SUMMARY.

1. *Anodonta grandis* and *Anodonta imbecillis* have been studied with reference to the glochidia and later stages in the life cycles.

2. Through experimental infestation, glochidia of *Anodonta imbecillis* have been reared under laboratory conditions through the period of transformation to the juvenile stage.

3. An examination of mature individuals collected at various times of the year yielded only glochidia, showing no evidences of transformation to the juvenile stage.

4. The claim that metamorphosis in *Anodonta imbecillis* occurs in the marsupia of the parent has not been confirmed by this investigation.

5. *Apomotis cyanellus* served as host to the glochidia of *Anodonta imbecillis*.

6. The transformation of glochidia of *Anodonta grandis* has been followed in experimental infestations using *Apomotis cyanellus* and *Amploplites rupestris* as hosts.

7. *Anodonta grandis* may be differentiated from *Anodonta grandis gigantea* on the basis of glochidial measurements.

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THE MORPHOLOGY AND PHYSIOLOGY OF THE SALAMANDER THYROID GLAND.

III. The Relation of the Number of Follicles to Development and Growth of the Thyroid in *Ambystoma maculatum*.

E. UHLENHUTH AND HILDA KARNS.

From the University of Maryland Medical School and the Marine Biological Laboratory, Woods Hole, Mass.[1]

Owing to the profound changes which the follicular pattern of the human thyroid gland undergoes in various pathological states, the follicular composition of the thyroid has been given careful attention by many students of the thyroid gland.

Martin Heidenhain was one of the first to examine the thyroids of various mammalian species with reference to the follicular pattern. He made the noteworthy discovery that profound differences exist between the different species of this group of animals, regarding the development and morphology of the follicular design of the thyroid. According to Heidenhain two types of follicular design must be distinguished, an association type to which belong the thyroids of the dog and cat, and a dissociation type to which belong the thyroids of the cattle and of man. In the association type the follicles which develop from the primary cell columns remain permanently in epithelial continuity, while in the dissociation type they become separated. In both types secondary "fusion" may take place. Thus in the thyroids of dog and cat secondary communication (canalization) between the adjacent follicles of an epithelial column may take place, if the quantity of secretion increases, and lead to the formation of tubuli possessing a continuous lumen. The same process may, at times, occur in man, at the stage of the primary cell columns, before splitting up into separate follicles has taken place; if it does occur, it acts inhibiting upon the

[1] Part of the work reported in this article was carried out with the aid of the "Julius Friedenwald Fund for Medical Research" of the University of Maryland.

process of splitting up. Heidenhain made some observations also on the growth and multiplication of follicles. He states that real budding of follicles is very rare; normally each cell of the primary cell columns is destined to develop sooner or later into one individual follicle. No new follicles, beyond the original number of cells, develop. In cattle, however, budding may be observed during the period of most rapid growth, just after birth. What appear to be buds in man, are only young follicles developing out of cells which failed to separate at the time when the other cells separated, the result of an incomplete dissociation. As to a multiplication of follicles Heidenhain observed that even in case of real buds these never become separated; likewise a cleavage of larger into smaller follicles does not occur.

In 1923 Williamson and Pearse (14, 15) published a study of the follicular pattern of the human thyroid gland and reported conditions essentially different from previous observations. According to these investigators the human thyroid is composed of gland-units a certain number of which make up one thyroid lobule. These units consist of an endothelial capsule the lumen of which is part of the general lymphatic system and communicates with other units and with the general lymphatic system, by means of a stalk-like lymph capillary. Very oddly in this lymphatic capsule are contained blood vessels, the capillaries of the gland-unit, which enter the capsule through the stalk-like hilus. In addition to the blood vessels each capsule contains probably one, possibly several coiled and freely floating epithelial columns. These epithelial columns of each gland unit, being the real glandular elements of the thyroid, are completely independent of the columns of other gland units, except for the lymphatic and blood-vascular communications. The solid epithelial columns represent the primary resting condition of the gland-unit. When functioning either of two completely different states—and only one of them at a time—may develop from these solid cell-columns. In both cases a lumen appears in the center of the cell column; in one case it becomes filled with colloid, in the other case with a more fluid substance. The colloid is not a secretion; the secretion is represented by the more liquid substance which, it is asserted, leaves the lumen of the epithelial

tube by way of "intracellular" tubules. Normally units in each of these three states are found together in one thyroid. But a unit which elaborates colloid cannot, at the same time, elaborate secretion. Before this is possible, it must revert to the original condition of the primary solid cell column.

So far these assertions, together with many others to be found in the publications referred to and adhered to by Williamson and Pearse in their more recent articles (16, 17) are mere statements; to support them the authors have furnished a surprisingly small number of facts. For reasons outlined specifically in a previous article we cannot subscribe to these theories at large; some of them are rather incoherent and others are based upon grossly wrong observations and interpretations. But in connection with the present article we are mostly interested to know whether in the human thyroid what impressed most observers as separate vesicles are in reality only parts of continuous tubules. It is well known and has been pointed out by Heidenhain and others that the human thyroid, in certain states, does seem to be composed of numbers of coiled, continuous tubules rather than of isolated vesicles.

Upon inspection of the thyroid of an exophthalmic goiter gland [1] we have convinced ourselves that within the larger lobules smaller areas can be distinguished, which are separated from the vicinity by finer connective-tissue strands; the follicles located within these areas suggest frequently that they might be merely parts of one larger, continuous tubule and sometimes resemble in a striking manner the condition found in *Ambystoma opacum*, illustrated in Fig. 39, Plate I. of a previous paper (11); as will be mentioned presently, in this species there really exists a condition somewhat similar to the one described by Williamson and Pearse.

Since 1922 the senior author of this article has been engaged in a study of the thyroid gland of the salamander *Ambystoma opacum;* the results of this work have been published in a number of previous articles (2 to 13). In this species of salamander the thyroid, during development, may follow either of the two types distinguished by Heidenhain (1) in mammals. But whichever

[1] This gland was received from Dr. A. S. Blumgarten, of the German Hospital in New York; it gives me pleasure to thank Doctor Blumgarten for his courtesy.

type it follows, the final result is, in the main, the formation of a large and coiled tube, distended with secretion to a varying degree in different places of its course, and resembling the condition described by Williamson and Pearse for the thyroid of man. The whole gland of the marble salamander may be compared, at that stage, with one single unit of the human thyroid gland. This continuous tube forms through a process of fusion of the smaller follicles, very much as described by Heidenhain, except that in case of previous dissociation really separated follicles fuse with each other. It was found that the development of this tube-like follicle, together with the elaboration and storage of the secretion product (a mixture of unstainable and, mainly, stainable colloid) coincides with the larval period. It represents, what was called, the developmental or storage phase of a functional cycle the second phase of which is the "functional phase" or phase of colloid release, which coincides with metamorphosis and is characterized, as regards the follicular pattern, by a complete collapse of the follicular tube, caused by the sudden release of the secretion product from the lumen of the follicles. The functional phase is followed again by the storage phase which now is initiated by an enormously increased elaboration of predominantly unstainable colloid. Like in man and other mammals—and unlike the assertions made by Williams and Pearse—the two secretion products, the chromophobe and the chromophile colloid, are always elaborated in and excreted from the same cells at the same time and into the same secretion-space, although in a varying proportion.

The important feature in the thyroid of the marble salamander and the one of especial interest in connection with the present article is the existence of a phase during which the small primary follicles fuse with each other to form a large tube, sometimes resembling a coiled tube, sometimes exhibiting a more bag-like appearance, which is distended with the secretion product of the cells. At the time when these observations were made the thyroid gland of *Ambystoma tigrinum* and of the axolotl, in addition to the thyroid of the marble salamander, were studied on sections and it was noticed that the follicular patterns of these glands differ markedly from that of the thyroid of the

marble salamander; the presence of a particularly large follicle was not noticed in these glands.

It was thought desirable to make a comparative study of the follicular pattern of several different species of salamanders, to see what was essential in the follicular pattern of the thyroid in general and whether differences in the follicular pattern corresponded to certain specific physiological differences of the thyroid apparatus. As at the same time large numbers of thyroids of experimental material had to be examined, the need was felt of developing a method which would enable us to make accurate examinations of the follicular pattern without the laborious work of sectioning and as soon as possible after the removal of the organs from the animals. It was found that upon maceration with 40 per cent. nitric acid the thyroid can be taken apart completely into its individual follicles, the size and shape of the follicles can be studied and the exact number of follicles can be ascertained for each individual thyroid gland. A brief account of the results thus obtained was published in the *Anatomical Record* (Uhlenhuth, 12).

In the present article the results obtained with this method for the thyroid of the salamander *Ambystoma maculatum* only will be reported; the thyroids of other species of salamanders have been successfully investigated with this method and publication of these investigations will be made soon. It should be mentioned here that the same method can be applied very successfully also to the thyroids of warm-blooded animals (dog) and of man.

Method.

The animals were anesthetized with chloretone (10 cc. of an 0.5 per cent. aqueous solution of chloretone plus 40 cc. of a 30 per cent. Ringer solution) and pinned up in a tray under 30 per cent. Ringer solution. The thyroids were removed in the usual way and placed at first into a dish containing a 30 per cent. Ringer solution. From this solution they were placed directly into a 40 per cent. nitric acid (Merk's Blue Label). It is best to leave them in the acid for from 12 to 24 hours and then to transfer them into a large dish filled with tap water. The water should be changed at least once. The glands treated in this fashion

should be dissected as soon as possible to avoid softening of the epithelial walls of the follicles. If this procedure is employed the connective tissue will be found disintegrated sufficiently to give way to the slightest pulling, while the follicles show great resistance and can be separated from one another without the least injury to the epithelial walls. The colloid assumes a yellowish tint, the cells are whitish. In case a part of the epithelial wall is scraped off or components belonging to one single follicle are torn asunder, the defect of the epithelium and the smooth, yellowish surface of the denuded colloid can be noticed at once.

Each thyroid was completely dissected into its individual follicles and the absolute number of follicles was ascertained (Table I., column 6). Representative follicles as to shape and size were then selected and their outlines drawn at a magnification of × 33 (Zeiss Binocular Dissection Microscope, Oc. 2, Obj. a_2).

In order to determine the relative number of the follicles, *i.e.* the number of follicles per unit of thyroid volume, it would have been necessary to determine the volume of each thyroid gland. As only in a few cases the thyroid volume was ascertained another, less accurate method was resorted to. Of each thyroid the largest optical section was drawn with the aid of a camera lucida, at a magnification of × 33, after the thyroids had been removed from the acid into the water. With the aid of a planimeter the area in cm^2. of each outline drawing was determined (Table I., column 8). By dividing the number of follicles over the area the number of follicles per cm^2. area was found for each thyroid (Table I., column 7). This method would give correct results only if the cross section of each thyroid were a circle. As some thyroids are less cylindrical than others, the values for the relative numbers of follicles as recorded here are only approximately correct. No conclusions, however, were drawn from these values beyond those suggested by the continuity and persistency of the change of this value with the progressing increase in the size of the thyroids and age of the animals.

Material.

The investigations reported in the present article were carried out on the species *Ambystoma maculatum*, in spring and summer

1925 and 1926. With the exception of the larvæ of the series CCLXII. and the sex-mature animals of the series CCLXX. the animals were reared from eggs in the laboratory. All animals were kept under the same conditions except where mention is made of the contrary. All animals of the same series are the offspring of the same female, except the animals of series CCLXII. and CCLXX. The entire material was collected by Mr. George Gray in the vicinity of Woods Hole, Mass.

The Number of Follicles.

1. Average number of follicles: The average number of follicles of the thyroid gland of the spotted salamander, as calculated from the thyroids of 88 normal animals examined since 1925, is 39.7 follicles. There is, however, a considerable variability from the average; the smallest number of the follicles in one thyroid was 5 follicles, the largest number 72 follicles (Table I., column 6).

Explanation to Table I.

The early larval stages (from No. 1 to No. 29) are arranged according to body length in mm.; the older larval stages and the metamorphosed animals are arranged according to stage, as during these periods of life the stage and not the size of the animal determines the condition of the thyroid gland.

The areas of the thyroids of the first seven animals (column 8) were drawn from the fresh gland, in all the other glands the areas were drawn after removal of the thyroids from the nitric acid into water. Comparison of the area before and after the acid treatment showed a shrinkage of the thyroid, which decreased the area by approximately 18 per cent. of the area of the untreated thyroid; therefore 18 per cent. should be subtracted from the values of the area of the first seven animals, to obtain the correct measurement.

Abbreviations.

E.pr.sl. ..... Eyes protrude slightly.
E.pr.d. ..... Eyes protrude distinctly.
E.pr.c. ..... Eyes protrude considerably.
Yell.N. ..... The even coloration of the earlier larval stages has been replaced by a yellow network on dark background.
Op.sh. ..... Opercular pouch, on ventral side, has started to grow to the body wall and has become shallow as compared to the previous larval stage.
G. ..... Gill slits.
Pieces 1 day ..... Cast off, for the first time, some small pieces of epithelium, 1 day before examination of thyroid.
Sh.Sk. ..... Shed his skin completely.
L. ..... Placed on land (moist filter paper).
Eats ..... Started taking food again.

TABLE I.

NORMAL *Ambystoma maculatum*, EXAMINED SINCE 1925.

Number.	Series.		Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
1	CCLXXI.	3	2.5 toes; 1 balancer left.	7.5	10	—	—	0.16
2	CCLXXI.	1	2.5 toes; no balancer left.	8.0	10	—	—	0.16
3	CCLXXI.	7	3.0 toes; no balancer left.	8.4	12	—	—	0.12
4	CCLXXI.	9	3.5 toes; hind limb buds out.	9.5	21	—	—	0.33
5	CCLXXI.	10	3.5 toes; hind limb buds out.	9.6	21	—	—	0.25
6	CCLXXI.	11	4.0 toes; hind limb buds split in 2.	9.6	28	—	—	0.41
7	CCLXXI.	12	6.0 toes.	11.1	29	—	—	0.14
8	CCXLVII.	32	9.0 toes.	13.4	126	*28*	80	0.35
9	CCLXXI.	14	8.5 toes.	15.1	44	*38*	42	0.9
10	CCXLVII.	33	9.0 toes.	16.4	126	*28*	62	0.45
11	CCLIV.	10	Eyes protrude slightly.	16.5	118	*33*	—	—
12	CCXLVII.	28	9.0 toes.	17.6	74	*32*	22	1.45
13	CCXLVII.	34	Larval.	17.9	126	*32*	35	0.95
14	CCLXXIV.	1	Larval.	20.0	65	*52*	43	1.2
15	CCLXXIV.	2	Larval.	21.3	66	*28*	22	1.3
16	CCLXXI.	16	Larval.	21.4	60	*34*	16	2.2
17	CCLXXI.	19	Larval.	21.5	63	*58*	25	2.3
18	CCLXXI.	20	Larval.	22.2	63	*24*	13	1.9
19	CCLXXI.	17	Larval.	22.8	62	*31*	13	2.5
20	CCLXXI.	18	Larval.	22.8	63	*38*	16	2.4
21	CCLXXX.	1	Eyes protrude slightly.	23.4	65	*50*	14	3.6
22	CCLXXX.*a*	5	Eyes protrude slightly.	23.8	76	*42*	26	1.65
23	CCLXXX.*a*	2	Eyes protrude slightly.	24.0	70	*52*	22	2.35
24	CCLXXI.*a*	1	Eyes protrude slightly.	25.7	75	*42*	17	2.55

[1] For changes of the color pattern before and after metamorphosis see Uhlenhuth, J. Experim. Zoöl., 1917, XXIV., 237–301 (p. 243).

[2] The number of follicles could not be ascertained as this thyroid consisted of a solid and continuous mass of epithelial cells, in which only four follicles were differentiated.

TABLE I. (*continued*).

Number.	Series.		Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
25	CCLXXI.*a*	2	E.pr.sl.; [1] larval color pattern.	27.9	77	*51*	16	3.1
26	CCLV.	1	E.pr.sl.	28.3	91	*37*	13	2.8
27	CCLXXIV.*a*	1	E.pr.distinctly; larval color patt.	29.2	92	*49*	14	3.4
28	CCLV.	2	E.protrude.	30.2	91	*25*	7	3.6
29	CCLXXIV.*a*	2	E.pr.distinctly; larval color patt.	32.3	92	*29*	7	4.2
30	CCLXXI.*b*	2	E.pr.sl.; yellow network.	27.2	79	*41*	14	2.9
31	CCLXXI.*a*	4	E.pr.d.; yellow network.	31.1	77	*35*	14	3.4
32	CCLXXI.*b*	3	E.pr.d.; yell.N.; Operculum shallow.	30.6	87	*56*	17	3.35
33	CCLXXI.*b*	5	E.pr.d.; yell.N.; Op. shall.	31.1	88	*72*	11	6.85
34	CCLXXIV.*a*	3	Pieces 1 day; E.pr.d.; yell.N.	33.7	96	*32*	11	2.85
35	CCLXXI.*a*	5	Pieces 1 day; E.pr.cons.; yell.N.; O.sh.	35.9	88	*35*	13	2.75
36	CCLXII.	6	Yellow N.	36.0	125	*10*	3	3.5
37	CCLXII.	4	Late larval; Eyes protrude.	37.3	125	*35*	7	4.7
38	CCLXII.	10	Late larval; Eyes protrude.	37.4	127	*30*	7	4.6
39	CCLXXI.*a*	6	E.pr.consid.; yell. spots; Op. and G. closed.	35.6	89	*65*	12	5.4
40	CCLXII.	8	Begins Sh.Sk.; Op.sh.; G. open.	37.3	126	*37*	8	4.65
41	CCLXII.	7	Begins Sh.Sk.; Op. very sh.; G. open.	42.0	126	*25*	3	7.45
42	CCLXXI.*b*	6	Just shedding; O. and G. partly open.	32.4	91	*47*	14	3.45
43	CCLXXI.*a*	3	Just shedding; Op. and G. closed.	27.8	77	*30*	10	2.95
44	CCLXII.	2	Just shed Sk.; Op. closed; G. partly op.	42.4	126	*31*	4	8.2
45	CCLXXI.*a*	12	Just shed Sk.; Op. and G. partly open.	—	91	*28*	9	3.05
46	CCLXXI.*b*	1	Just shed Sk.; Op. and G. closed.	27.1	77	*43*	17	2.5
47	CCLXII.	5	Sh.Sk. ½ hour; Op. and G. nearly closed.	40.1	125	*5*	1	4.6
48	CCLXII.	9	Sh.Sk. 3 hours.	37.0	126	*44*	8	3.5
49	CCLXXIV.	3	Sh.Sk. 0 days.	22.5	68	*48*	19	2.5

TABLE I. (*continued*).

Number.	Series.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
50	CCLVII. 1	Sh.Sk. 0 days; Op. closed; G. open.	33.0	95	*63*	16	4.0
51	CCLXXI.*a* 14	Sh.Sk. 0 days; Op. and G. nearly closed.	35.2	95	*24*	8	3.2
52	CCLIX. 6	Land 0 days; Op. and G. closed.	30.7	120	*33*	11	3.0
53	CCLXXIV.*a* 5	Sh.Sk. and L. 1 day; G. closed; Op. very sh.	—	106	*26*	10	2.5
54	CCXXXVI. 9	Sh.Sk. and L. 1 day; Op. and G. closed.	29.9	118	[2]		2.4
55	CCLVI. 1	Sh.Sk. 1 day; Op. closed; G. open.	35.6	91	*52*	10	5.5
56	CCLVII. 5	Sh.Sk. and L. 1 day; Op. and G. closed.	27.8	123	*33*	10	3.35
57	CCLXII. 11	Sh.Sk. and L. 1 day; Op. and G. closed.	36.1	128	*30*	7	4.3
58	CCLXII. 12	Sh.Sk. and L. 1 day; Op. and G. closed.	32.4	128	*37*	10	4.65
59	CCLXXI.*a* 7	Sh.Sk. and L. 1 day; Op. and G. closed.	33.8	89	*50*	12	4.2
60	CCLXII. 1	Sh.Sk. 2 days; L. 1 day; Op. and G. closed.	40.9	120	*39*	5	7.2
61	CCLXXI.*a* 9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11	4.6
62	CCLXXI.*b* 7	Sh.Sk. and L. 2 days; Op. and G. closed.	31.7	92	*71*	16	4.5
63	CCLXXI.*a* 8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	5.2
64	CCLXXI.*a* 13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6	7.9
65	CCLXII. 13	Sh.Sk. 3 days; L. 2 days; Op. and G. closed.	34.8	130	—	—	6.2
66	CCLXXI.*b* 8	Sh.Sk. and L. 3 days; Op. and G. closed.	28.6	95	*67*	21	3.2
67	CCLXXI.*a* 10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	4.25
68	CCLXII. 3	Sh.Sk. 4 days; L. 3 days; Op. and G. closed.	39.0	124	*54*	—	—
69	CCLXII. 14	Sh.Sk. 4 days; L. 3 days; Op. and G. closed.	41.4	130	*36*	4	8.15
70	CCLXXI.*b* 4	Sh.Sk. and L. 4 days; G. absorbed.	28.8	87	*32*	17	2.35
71	CCLXXI.*a* 11	Sh.Sk. and L. 4 days; G. absorbed.	29.6	89	*43*	10	4.45
72	CCLXXIV.*a* 4	Sh.Sk. and L. 5 days; Eats 1 day.	35.4	100	*47*	16	2.95
73	CCLXXI.*a* 15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7	5.7
74	CCLXXI.*a* 18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15	4.2

TABLE I. (*continued*).

Number.	Series.		Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
75	CCLXXI.*a*	19	Sh Sk. and L. 11 days. Eats 1 day.	33.1	101	*36*	15	2.45
76	CCLXXI.*a*	20	Sh.Sk. and L. 12 days. Eats 4 days.	34.9	101	*50*	9	5.4
77	CCLXXI.*a*	16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12	3.05
78	CCLXXI.*a*	17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10	4.45
79	CCLXXI.*a*	21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6	4.5
80	CCLXXI.*a*	22	Sh.Sk. and L. 17 days. Eats 8 days.	35.3	102	*47*	14	3.6
81	CCLXXI.*a*	23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25	2.9
82	CCLXXI.*a*	24	Sh.Sk. and L. 189 days; killed February.	40.5	279	*39*	10	4.1
82*a*	CCLXXI.*a*	25	Sh.Sk. and L. 197 days; killed April.	46.0	328	*36*	10	3.5
83	CCXXXIV.	12	Sh.Sk. 379 days; killed August.	51.5	474	*23*	7	6.6
84	CCXXXIV.	4	L. 403 days; killed August.	49.0	476	*24*	6	4.15
85	CCXXXIV.	13	Sh.Sk. and L. 539 days; killed February.	53.5	649	*11*	2	4.96
86	CCXXXVI.	10	L. 532 days; killed February.	42.5	650	*34*	7	4.9
87	CCXXXVI.	12	Sh.Sk. and L. 532 days; killed February.	54.2	657	*26*	8	3.3
87*a*	CCXXXIX.	10	L. 591 days; killed April.	67.0	703	*40*	3	11.2
88	CCLXX.	1	Adult female; killed May 1926.	103.5	—	*34*	3	11.2
89	CCLXX.	2	Adult female; killed May 1926.	93.0	—	*53*	5	11.0
90	CCLXX.	3	Adult female; killed May 1926.	88.0	—	*24*	3	8.6
91	CCLXX.	4	Adult female; killed May 1926.	101.3	—	*30*	2	13.6
92	CCLXX.	5	Adult male; killed August 1926.	86.2	—	*48*	3	15.5
93	CCLXX.	6	Adult male; killed August 1926.	83.5	—	*33*	3	10.8
94	CCLXX.	7	Adult female; killed Febr. 1927.	90.3	—	*52*	5	9.6
95	CCLXX.	8	Adult female; killed March 1927.	84.3	—	*59*	6	9.2
			Average			39.7	13.3	

The difference of the number of follicles is considerable not only between the thyroids of different individuals, but also between the two thyroids of the same individual (Table II.*a*).

TABLE II.*a*.

DIFFERENCES IN THE NUMBER OF FOLLICLES BETWEEN THE TWO THYROIDS OF THE SAME INDIVIDUAL.

Series Number of Animal.	Left Thyroid.		Right Thyroid.	
	Number of Follicles.	Area of Largest Optic Section of Thyroid in cm².	Number of Follicles.	Area of Largest Optic Section of Thyroid in cm².
1. CCLXXI.*a* 23	72	2.9	50	3.2
2. CCLXXI.*a* 24	39		32	
3. CCXXXIV. 13	11	5.0	19	6.5
4. CCXXXVI. 10	34	4.9	37	4.2
5. CCXXXVI. 12	26	3.3	23	2.8

2. Relation of the number of follicles to the size of the gland: The differences in the number of follicles are independent of the differences in the size of the glands. The number of follicles is not determined by the size of the gland. This fact may be ascertained by direct observation. It is also evident from comparison of the number of follicles with the values of the area of the thyroids. One thyroid, for instance (No. 1 in Table II.*a*) measured 2.9 cm²., but contained 72 follicles, while the thyroid of the other side of the same animal contained only 50 follicles, although it measured 3.2 cm².

In Table II.*b* are recorded the number of follicles, the thyroid-volume [1] and the number of follicles per cm³. of thyroid-volume for both thyroids of each of two animals. It will be noticed

[1] The thyroid-volume was calculated in the following manner. The largest and the smallest longitudinal optical sections of each gland were outlined and the areas measured as before. The area of the largest longitudinal optical section was multiplied by the largest diameter of the smallest longitudinal optical section and the area of the smallest longitudinal optical section multiplied by the largest diameter of the largest longitudinal optical section. The two products were added and the sum was divided over 2.

that the left thyroid of animal No. 1 contained fewer follicles than the right one, yet the volume of the left gland was larger than that of the right gland. In animal No. 2 of Table II.*b* the

TABLE II.*b*.

DIFFERENCES BETWEEN THE TWO THYROIDS OF THE SAME INDIVIDUAL IN RESPECT TO THE ABSOLUTE NUMBER OF FOLLICLES AND TO THE NUMBER OF FOLLICLES PER CM³. OF THYROID VOLUME.

Series Number of Animals.	Left Thyroid.			Right Thyroid.		
	Number of Follicles.	Volume of Thyroid in cm³.	Number of Follicles per cm³. of Thyroid Volume.	Number of Follicles.	Volume of Thyroid in cm³.	Number of Follicles per cm³. of Thyroid Volume.
1. CCXXXIV. 13.....	11	7.16	1.5	19	11.3	1.8
2. CCXXXVI. 12.....	26	4.2	6.2	23	3.2	7.1

larger thyroid contained more follicles, absolutely, than the right one, but relatively the number of follicles was smaller in the left one than in the right one.

The variability of the number of follicles of the thyroids of different animals will become evident from an inspection of Table I. There are a number of thyroids among the smallest ones (less than 3 cm². area), which are composed of over 50 follicles, while among the largest thyroids there is, for instance, one which measures 13.6 cm²., but contains only 30 follicles, and another one measuring 8.6 cm². and containing 24 follicles.

In Table II.*c* the absolute number of follicles, the thyroid-volume and the number of follicles per cm³. of thyroid-volume are recorded. As will be noticed, the variability of the number of follicles, independent of the size of the gland, is not a merely apparent one caused by an inexactness of the method of expressing the size of the thyroid by the area of the largest optical section; a similar variability is noticeable, if the size of the thyroid is expressed in volume.

3. Relation of the number of follicles to the growth of the thyroid: From an inspection of Table I. it will be obvious

that, although the variability of the number of follicles is considerable, it does not show any relation to the growth of the gland. In a general way the number of follicles remains unchanged during the entire life of the animal. This circumstance is born out most clearly by the values for the relative number of follicles (Table I., column 7). The relative number of follicles is at first high (80, 42, 62), decreases rapidly with the size of the gland and in adult animals is found to be between 2 and 5.

TABLE II.*c*.

VARIABILITY OF THE THYROID GLAND IN RESPECT TO THE ABSOLUTE NUMBER OF FOLLICLES AND TO THE NUMBER OF FOLLICLES PER CM³. OF THYROID VOLUME.

Series Number of Animal.	Left Thyroid.			Approximate Age of Animal.
	Number of Follicles.	Thyroid Volume in cm³.	Number of Follicles per cm³. of Thyroid Volume.	
1. CCLXXI.*a* 24	39	5.3	7.4	1st year
2. CCLXXI.*a* 25	36	4.7	7.7	
3. CCXXXIV. 13	11	7.2	1.5	2d year
4. CCXXXVI. 10	34	7.9	4.3	
5. CCXXXVI. 12	26	4.2	6.2	
6. CCXXXIX. 10	40	21.7	1.8	3d year
7. CCLXX. 7	52	23.7	2.2	Adult
8. CCLXX. 8	59	21.3	2.8	

The thyroid gland of the spotted salamander does not grow by multiplication of its follicles, but by an increase in the size of its individual follicles.

There is, in this species, no definite relation also between the number of follicles and the development of the gland, as will be discussed presently.

SIZE AND SHAPE OF THE FOLLICLES.

1. Types of follicles: The size and shape of the follicles shows an extreme degree of variation; nevertheless a number of general types of follicles can be distinguished.

In a general way it may be stated that the shape of the follicles is the more irregular the larger the follicles are.

(*a*) The smallest follicles (primary follicles) are usually round and in most thyroids strictly spherical (Fig. 1, *a*). But in nearly every thyroid there is one or several large follicles which are fairly round, possessing no diverticula at all (Fig. 1, *b* and *c*).

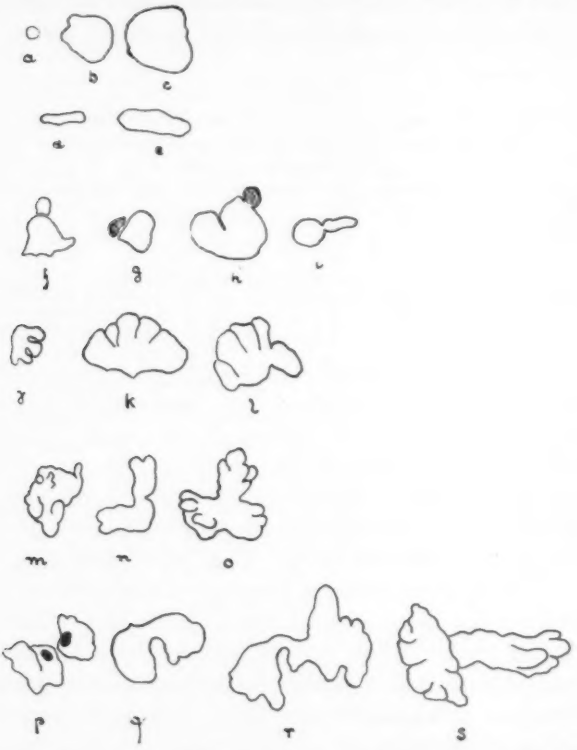


FIG. 1. Various types of follicles found in the thyroid gland of *Ambystoma maculatum*. Outlines drawn with the aid of a camera lucida, at a magnification of × 33 (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2).

The cross-lined areas indicate solid follicles.

(*b*) Another kind of follicles with smooth surface are the elongate, tube-like follicles of cylindrical shape (Fig. 1, *d* and *e*).

(*c*) The simplest type of diverticulated follicle is represented

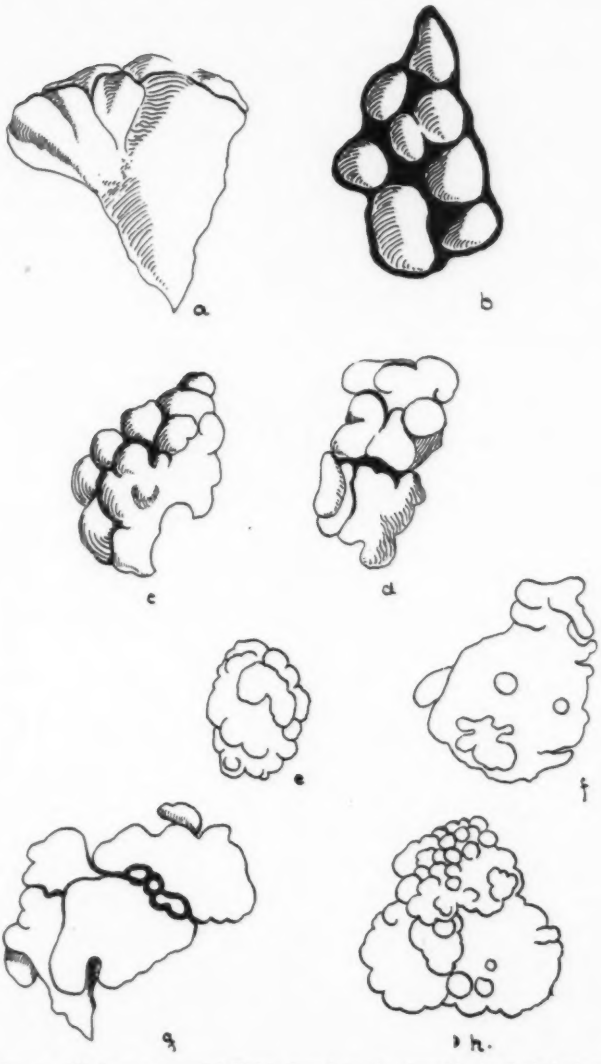


FIG. 2. Various types of follicles, found in the thyroid gland of *Ambystoma maculatum*. Drawn with the aid of a camera lucida, at a magnification of × 90 (Zeiss Binocular Dissecting Microscope, Oc. 4, Obj. a_3). *a*, side view; *b*, view of the base of the same follicle; *c*, side view; *d*, view of the base of the same follicle. *f*, a pollicle taken from the thyroid of *Notophthalmus torosus* (CCXLVI. 2).

by fairly large, round follicles bearing on their surface one or several bud-like, spherical diverticula of the same size as the spherical primary follicles (Fig. 1, *h* and *i*). This type is rare in the spotted salamander, but is found frequently in the tiger salamander as will be shown in a subsequent paper.

(*d*) A very conspicuous type is represented by follicles of conical or pyramidal shape (Fig. 1, *j*, *k*, *l* and Fig. 2, *a* and *b*). The apex tapers into a point; from the base project two or more finger-like diverticula. In the simplest case these follicles consist of two component follicles communicating at the apex by a small hole and diverging towards the base. But usually the distance along which the component follicles are fused is much more considerable; a conical follicle results, which, at the base, is split into two finger-like processes. In many thyroids, especially noticeably so in the thyroids of sex-mature adults, a dozen or more finger-like diverticula may project from the base, while the apex is still single and pointed (Fig. 1, *k* and Fig. 2, *a* and *b*).

(*e*) Another type of follicles, especially frequent in animals metamorphosed for some time and in adults, is represented by follicles bearing so many diverticula that their shape has become completely irregular (Fig. 1, *m* and *n*, Fig. 2, *c*, *d*, *e* and *f*).[1]

(*f*) While in each of the previous types one major follicle is recognizable, bearing on its surface a varying number of smaller follicles, there is another type, the "composite follicle," which consists of two or more equally large major component follicles (Fig. 1, *o* to *s*, Fig. 2, *g* and *h*). The component follicles may communicate with one another along a considerable area of their circumference (Fig. 1, *o* and *s*, Fig. 2, *g* and *h*), or communication may be established merely by a tiny hole (Fig. 1, *p*, *q* and *r*). Particularly interesting are those follicles whose component follicles are in close apposition with one another along a considerable portion of their entire circumference, yet communicate only by a very small hole (Fig. 1, *p*) or by a short stalk. Each of the component follicles may possess a relatively smooth surface or may bear, in its turn, numerous smaller diverticula

[1] *f* has been taken from the thyroid of *Notophthalmus torosus*, but is representative for many follicles of the spotted salamander.

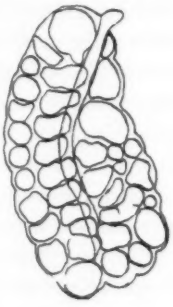
on its surface. In the latter case extreme irregularity and complexity of the follicle results.

(*g*) The smaller follicles of each type may be without a lumen, consisting of a solid mass of cells. Solid follicles are found more often among the spherical primary follicles than among the other types. In the large follicles one or several diverticula may be found solid (Fig. 1, *h*).

(*h*) Not infrequent are flat follicles; they still contain some colloid, but the lumen is very small, slit-like.

2. General arrangement of the follicles: Sometimes no definite design can be found in the arrangement of the follicles. But in most thyroids the follicles exhibit very definite relations.

The follicles are frequently arranged in perfect rows which follow the course of the main vessel (external jugular vein); the original columnar arrangement which the thyroid shows before the differentiation into follicles may be preserved thus in the thyroid of the adult (Fig. 3).



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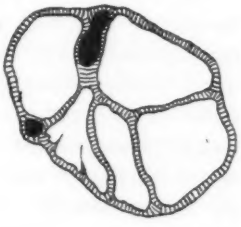

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FIG. 3. Diagram of an entire thyroid gland (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2; magnified × 33), showing the arrangement of the follicles in rows parallel to the external jugular vein.

FIG. 4. Diagram of a cross section through a thyroid gland (Zeiss Binocular Dissecting Microscope, Oc. 4, Obj. a_2; magnified × 90), showing the arrangement of the follicles. White, follicles; cross lined, connective tissue; black, large veins.

The follicles are frequently so arranged, within the cross-section of the organ, that each follicle not only borders, with its central extremity, on one of the large vessels, but also comes to lie, with its peripheral surface, within the periphery of the whole organ (Fig. 4).

At each (caudate and craniate) end of the thyroid is usually one particularly large, fairly round follicle. The small spherical follicles—which in *A. maculatum* are always scarce—are usually located in the periphery of the organ (in *A. tigrinum* some thyroids contain as many as 25 per cent. extremely small follicles; here they are often crowded together into nests surrounded on all sides by larger follicles).

Characteristic is the location of the large, finger-like follicles. The apex of these follicles is always located in the center of the cross-section of the gland (Fig. 4). The finger-like diverticula point towards the periphery and their free ends come to lie, with their entire surface, within the periphery of the gland.

3. Relation of size and shape of follicles to growth and development of the thyroid: While the number of follicles bears no relation to either growth or development of the thyroid, the size and shape of the follicles is distinctly correlated to the size of the gland and, therefore, indirectly to the developmental stage of the gland.

The development of the thyroid of the spotted salamander, during the very early stages, up to the completion of the limb development, is very similar to the development of the thyroid in the marble salamander, as described in a previous paper (11). If the entire organ is removed and examined in fresh condition under the microscope, it will be seen that it consists, at first, of a solid mass of cells, devoid of any orderly arrangement of the cells. Soon this mass of cells assumes a columnar shape. The column is at first a continuous mass of epithelial cells. Gradually it becomes differentiated into small spherical groups of epithelial cells and finally each of these groups develops a lumen in its center, and differentiates into a primary follicle. At that stage maceration in nitric acid was attempted, but was unsuccessful, in as much as separation of the primary follicles was impossible. Microscopical examination shows that the connective tissue has not yet invaded the epithelial anlage; the follicles are not yet separated by connective tissue, but are in perfect epithelial continuity with each other. The youngest animal in which isolation of the follicles was partly successful was animal No. 9 in Table I., a larva possessing nearly the

complete number of toes. The right thyroid of this animal was isolated into 38 portions. Of these 29 were recognized to be small individual spherical globules; although, as microscopical examination of the left thyroid showed, these globules contained a distinct lumen, the contents must have been different from the colloid of later stages, as it did not turn yellow in the nitric acid. Eight other portions of the same thyroid were of irregular shape and consisted probably partly of primary follicles and partly of solid cell-groups, all of which were connected directly by epithelial tissue. The last portion was quite large constituting a considerable part of the entire organ and being partly columnar in shape, representing in its entirety a solid mass of epithelial cells (Fig. 5, *A*).

The youngest larvæ in which complete isolation of the follicles is possible measure about 20 mm. body-length (No. 14 in Table I.) and are in the possession of completely developed toes. In the thyroids of these animals only a small number of spherical primary follicles is present (about 10 to 15 per cent.), the other follicles vary in size up to five times the size of a primary follicle and are slightly irregular in outline (Fig. 5, *B*).

By the time metamorphosis approaches (Fig. 5, *C*) the smallest follicles are still of the size of primary follicles, they are almost invariably spherical or at least roundish and their relative number is unchanged (10 to 15 per cent.). The largest follicles have not only increased considerably in size, but have become irregular in shape; among them may be represented all the types enumerated previously. Between these two extreme types of follicles transitional forms are found.

About at the time of the first skin shedding flat follicles containing almost no colloid and corresponding to the collapsed follicles found regularly in *A. opacum* after the first skin shedding are conspicuous; an exact study of that condition has not been possible with the maceration method.

The follicular pattern as found in the late larval stages remains almost unchanged during the entire life of the individual. In the adult animal the largest follicles are still larger and more irregular. Among the smallest follicles there may be found, in the first and second year, still one or several small, spherical

FIG. 5. Four thyroid glands of different developmental stages of *A. maculatum*, showing the changes of the follicles in size and shape. *a*, outlines of the largest optical section of each thyroid gland; below the outlines of representative follicles of each gland. Cross-lined areas indicate a solid cell mass. Outlines drawn with the aid of a camera lucida, at a magnification of × 33 (Zeiss Binocular Microscope, Oc. 2, Obj. a_2).

A. Thyroid of a larval animal (No. 9 in Table I.) of 43 days of age and 15.1 mm. body length.

B. Thyroid of a larval animal (No. 14 in Table I.) of 65 days of age and 20.0 mm. body length.

C. Thyroid of a larval animal (No. 29 in Table I.) of 92 days of age and 32.3 mm. body length, approaching metamorphosis.

D. Thyroid of an adult, sex-mature male (No. 92 in Table I.) of 86.2 mm. body length (age unknown).

or at least roundish follicles of the size of a primary follicle. In the sex-mature adults, however, the smallest follicles are larger than the primary follicles and may be five or more times as large as a primary follicle (Fig. 5, *D*).

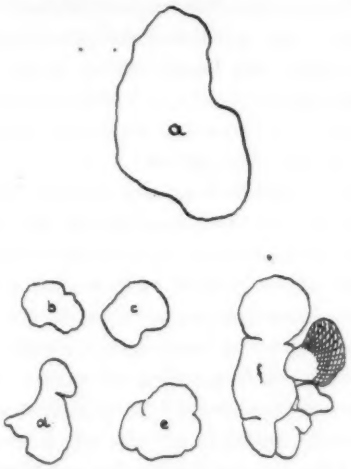

FIG. 6. Thyroid and follicles composing it, of specimen No. 47 in Table I., an animal which was examined one half hour after the first skin shedding. The whole thyroid gland was composed of only five follicles, one especially large and making the largest part of the entire organ.

Outlines drawn with the aid of a camera lucida, at a magnification of × 33 (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2).

a, outlines of largest optical section of the whole thyroid gland.

b, *c*, *d*, *e* and *f*, outlines of the follicles composing the gland.

THE MODE OF THYROID GROWTH.

As may be calculated from the values recorded in column 8 of Table I. (area in cm^2.) the volume of the adult thyroid gland of *A. maculatum* is about 680 times as large as the volume of the thyroid of a larva at the time at which the first toes are developed.

This considerable growth might take place in two ways, either by an increase in the size of the follicles or by an increase in the number of the follicles.

The diverticulation of the follicles, increasing with the size of the gland, the presence of follicles which consist of several

component follicles communicating often only by a narrow stalk and finally the presence of relatively very small follicles in the thyroids of practically every stage, if taken together, would suggest that the follicles develop buds which, after attaining a certain size, separate from the mother follicles. If this assumption were correct, the number of follicles should increase with the size of the gland and more follicles should be found in the adult than in the larval gland. In reality the number of follicles stays unchanged. *The growth of the thyroid is effected entirely by an increase in the size of the follicles.*

Nevertheless it could be imagined that the follicles composing a particular thyroid at a certain stage are not the same follicles composing the same thyroid at another stage. The follicles could divide continuously without increasing in number, if just as many follicles disintegrate as new ones are forming. Although under certain experimental conditions, as will be described in a subsequent article, disintegration of many follicles may be produced, positively no atrophic or degenerating follicles were ever found in the normal thyroid of *A. maculatum*. This observation is fully in accord with the condition found through histological study in the thyroid of *A. opacum*.

Although the shape of the follicles suggests strongly a process of budding and cleavage of larger into smaller follicles, it is obvious that in reality *separation of the bud-like diverticula from the mother follicles and cleavage of larger follicles into smaller follicles does not take place.*

The presence of bud-like diverticula and of composite follicles may be explained in two different ways. The diverticula may form as an outgrowth from the wall of the original follicles or they may be the result of fusion of smaller follicles with larger follicles. Histological study of the thyroid of *A. opacum* showed convincingly that follicular fusion plays a very important rôle in the growth of the thyroid of this animal. Conditions in *A. maculatum* are somewhat less favorable to analyzing this phase of the problem. Several facts, however, suggest, that in the spotted salamander processes of follicular fusion are going on during a large part of the animal's life. As pointed out, small spherical follicles are present in the thyroids of almost

every stage. A complete analysis of the problem hinges around the question whether the primary follicles found in any particular stage of an individual thyroid gland are the same as those present at a later stage or whether new primary follicles are developing continuously during the growth of the thyroid. The primary follicles retain practically the same size from an early larval stage up to the winter of the second year of the animal's life. If the primary follicles found during the second winter are the same as those present at the early larval stages, it would mean that they have not been taking part in the general follicular growth. It would be difficult to explain this peculiarity. Neither pressure nor lack of nourishment can be invoked as the cause of it; the primary follicles are located almost invariably at the periphery of the organ and are frequently in close vicinity of large vessels. Moreover the primary follicles do not by any means lack the potential ability of growing; on the contrary they may finally attain a considerable size as may be seen in the thyroids of completely adult, sex-mature animals.

If the primary follicles seen in the thyroid during the later stages are not the same individuals as those seen at the earlier stages, then it would mean that new primary follicles are forming continuously during the growth of the thyroid, but do not increase in number, because a corresponding number fuse with the follicles already present, either while they are still small and spherical (bud-like diverticula) or after they have reached a larger size (composite follicles). Two facts were observed, which would fit in well with this interpretation. As has been mentioned above, follicles are found frequently, which consist of several large component follicles communicating with each other sometimes merely by a small hole. Since cleavage of follicles does positively not occur, this condition may indicate a fusion in its earliest beginning. Particularly suggestive are such follicles which are in closest apposition along a considerable surface, but communicate nevertheless only by a small opening in the adjacent walls (Fig. 1, *p*). The small spherical follicles present very frequently a relation to the large follicles, which is very similar to the relation of the bud-like diverticula and is different from it only by the lack of communication (Fig. 1, *f*).

The other circumstance of interest in this connection is the frequent presence of tiny solid cell masses in thyroids of nearly every stage. These cell masses are difficult to find in the macerated glands and it may be for that reason that they have been overlooked in many glands. With the method of maceration a minute analysis of these cell masses is impossible. It should be pointed out, however, that they are epithelial in nature, distinctly different from the connective tissue as well as from the clusters of red blood corpuscles. It is believed that they correspond to similar cell masses found in histological thyroid sections of *A. opacum* and called "reserve cell masses" in a previous paper (11). Microscopical study of these cell masses in the marble salamander showed that they are the source for continuous development of small young follicles. Most probably the same condition prevails in the spotted salamander; the reserve cell masses split off, during a large part of the animal's life, new primary follicles which in turn fuse with the old follicles. The increase in the size of the smallest follicles as observed in the glands of adult animals indicates that in these glands development of new primary follicles has ceased and no further fusion of the older primary follicles with the large follicles takes place.

These facts suggest that *at least a certain number of diverticula are the result of fusion of smaller follicles with larger ones.*

It was observed that in *A. opacum* intracellular colloid globules may enlarge within the cells of the follicular wall, come to lie free within the epithelial wall and finally fuse with the main mass of intrafollicular colloid (11). While they lie free in the epithelial wall they may, sometimes, form a small, bud-like protrusion on the outside of the follicle. Possibly some of the very smallest diverticula in *A. maculatum* are formed by such intracellular colloid droplets.

It is of interest to note that Heidenhain found separation of buds from the mother follicles and cleavage of large into small follicles entirely absent in the thyroid of mammals. Our findings in the thyroid of *A. maculatum* corroborate entirely Heidenhain's observation in the thyroid of mammals. As mentioned above, Heidenhain distinguishes two types of thyroids, an association and a dissociation type. The thyroid of *Ambystoma maculatum*

belongs to the dissociation type. The separation of the follicles, however, is absolutely complete. The bud-like diverticula of the large follicles, if they occur at all, are, according to Heidenhain, primary follicles not yet isolated. In *A. maculatum* the bud-like diverticula cannot be interpreted this way; as indicated by the perfectly smooth and spherical shape of the primary follicles isolation takes place at an early stage.

Fusion of follicles isolated previously was not observed by Heidenhain in the mammalian thyroid gland; it takes place, according to this author, only between the lumina of follicles which have not been separated before. As described in a previous article (11) this kind of fusion is not uncommon in the marble salamander. In the spotted salamander, it seems, fusion is accomplished between follicles which have been really separated before. We are in doubt, however, that in the material and with the method used by Heidenhain a secondary fusion of previously separated follicles could be detected, if it did exist.

Epithelial reserve cell masses from which follicles are split off continuously were not observed by Heidenhain in the mammalian gland. He considers the small follicles of the cattle thyroid as the primary result of the general dissociation taking place at a very early stage. According to his views large numbers of the small primary follicles remain permanently unchanged, from the time of their separation till old age. In *A. punctatum* these primary follicles are absent in the adult glands indicating that finally the primary follicles begin to grow too.

Exceptional Cases of the Follicular Pattern.

In *A. opacum* the follicular pattern shows a very definite relation to metamorphosis. Two types of follicular design are characteristic. As the follicles begin fusing with each other shortly after the completion of the limb-development, the thyroid consists, some time before metamorphosis takes place, for the most part of a large, tube-like follicle distended greatly with colloid. The other characteristic pattern is found just after the first skin shedding. At this time the large, tube-like follicle collapses nearly in its entire extent, as the colloid is released from its lumen, and then forms a nearly solid and continuous mass of cells.

As will be evident from the facts described above neither of these conditions was found to be an integral part of the developmental cycle of the thyroid of *A. maculatum*. Nevertheless we found two thyroids which recall in a most conspicuous manner the structure characteristic for the thyroid of *A. opacum*.

The first case is represented by an animal of series CCLXII., animal No. 47 in Table I.; its thyroid was examined one half hour after the first skin shedding. Only one of the two thyroids was macerated. It consisted of only 5 follicles (Fig. 6). Small spherical follicles were missing entirely. Four of the five follicles were of good size, but did not show anything unusual. The fifth follicle, however, was excessively large, making up the largest portion of the entire gland. It consisted of seven component follicles of rather smooth outlines; one of these was partly solid. On the whole this follicle resembled the large bag-like follicle found normally in the thyroid of *A. opacum* and developing there by fusion of the smaller follicles. In *A. maculatum* this condition was found only once among 120 animals.

The second case is represented by the thyroid of animal No. 54 in Table I., which was killed one day after the first skin shedding. This thyroid consisted almost in its entirety of a solid mass of cells, devoid of any lumen and colloid. In addition 11 smaller masses were isolated. But only 4 of them were follicles of vesicular nature; the other seven were solid cell masses; it was suspected that they had been broken off, in the process of dissecting the macerated gland, from the main mass. This thyroid resembles closely the condition of the thyroid found normally in *A. opacum* shortly after the first skin shedding and called in a previous paper (11) "stage of colloid release." In *A. maculatum* it was found only once among 120 animals. But in another species, *A. jeffersonianum*, of which only few thyroids were examined by the maceration method, exactly the same condition was met with in an animal which was examined one day after it had been placed on land (CCXXXIII. 7). Animals of the same series from which the *A. maculatum* described above had been taken were examined more than one year after the first skin shedding; none of them showed this condition of

almost complete continuity of the epithelium and lack of colloid. It is therefore certain that it was not a peculiar thyroid structure characteristic for the offspring of one particular female.

It may be said, then, that although as a rule the follicular pattern of the thyroid of *A. maculatum* does not show any striking changes corresponding to certain functional changes, such changes may develop in rare cases. They resemble the changes taking place regularly in *A. opacum*. It should be noted especially that while the development of one particularly large tube-like follicle is not an integral phase in the functional cycle of the thyroid of the spotted salamander, it does occur occasionally.

On the whole the differences of the follicular pattern of the thyroid, existing between two so closely related species as *A. maculatum* and *A. opacum*, are very striking. Yet the occurrence of the two exceptional cases of follicular pattern in *A. maculatum* shows, that in the thyroid of this species the ability of developing the follicular pattern of *A. opacum* is potentially present.

Comparison of the thyroids of the two species of salamanders examined furnishes, we believe, further evidence to show that the plasticity of the endocrine system is very considerable in the group of amphibians.

Variability of the Number of Follicles.

Owing to the extreme variability of the number of follicles it is difficult to determine whether or not a change in the number of follicles can be effected by any particular experimental procedure. Nevertheless, as we have seen in experiments to be described in a subsequent paper (13), it is possible to change so considerably the number of follicles, that the degree of the change exceeds the degree of variability. In the present article we will discuss only such variations the causes of which are not entirely understood.

As was pointed out, when reference was made to Table I. the number of follicles varies between 5 and 72, if all of the animals examined are included. If, however, animals of the same parentage are compared, the variability is considerably less, as may be seen from an inspection of Table III. All animals

TABLE III.

Series CCLXXI.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Area, in cm.³ of Largest Optical Section of Thyroid at a Magnification of × 33.
3	2.5 toes; 1 balancer left.	7.5	10	—	—	0.16
1	2.5 toes; no balancer left.	8.0	10	—	—	0.16
7	3.0 toes.	8.4	12	—	—	0.12
9	3.5 toes; hind limb buds.	9.5	21	—	—	0.33
10	3.5 toes; hind limb buds.	9.6	21	—	—	0.25
11	4.0 toes; hind limb buds split in two.	9.6	28	—	—	0.41
12	6.0 toes.	11.1	29	—	—	0.14
14	8.5 toes.	15.1	44	*38*	42	0.9
16	Larval.	21.4	60	*34*	16	2.2
19	Larval.	21.5	63	*58*	25	2.3
20	Larval.	22.2	63	*24*	13	1.9
17	Larval.	22.8	62	*31*	13	2.5
18	Larval.	22.8	63	*38*	16	2.4
a 1	E.pr.sl.	25.7	75	*42*	17	2.55
a 2	E.pr.sl.	27.9	77	*51*	16	3.1
a 4	E.pr.d.;[1] yell.N.	31.1	77	*35*	15	3.4
a 5	Pieces 1 day; yell.N.; Op. very shall.	35.9	88	*35*	13	2.75
a 6	Yellow spots; Op. and G. closed.	35.6	89	*65*	12	5.4

TABLE III. (*continued*).

Series CCLXXI.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Area, in cm.² of Largest Optical Section of Thyroid at a Magnification of × 33.
a 3	Just shedding Sk.; Op. and G. closed.	27.8	77	*30*	10	2.95
*a*12	Just Sh.Sk.; Op. and G. partly open.	—	91	*28*	9	3.05
*a*14	Sh.Sk. 0 days; Op. and G. nearly closed.	35.2	95	*24*	8	3.2
a 7	Sh.Sk. and L. 1 day; Op. and G. closed.	33.8	89	*50*	12	4.2
a 9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11	4.6
a 8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	5.2
*a*13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6	7.9
*a*10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	4.25
*a*11	Sh.Sk. and L. 4 days; Gills absorbed.	29.6	89	*43*	10	4.45
*a*15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7	5.7
*a*18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15	4.2
*a*19	Sh.Sk. and L. 11 days; Eats 1 day.	33.1	101	*36*	15	2.45
*a*20	Sh.Sk. and L. 12 days; Eats 4 days.	34.9	101	*50*	9	5.4
*a*16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12	3.05
*a*17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10	4.45
*a*21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6	4.5
*a*22	Sh.Sk. and L. 17 days.	35.3	102	*47*	14	3.6
*a*23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25	2.9
*a*24	Sh.Sk. and L. 189 days; killed February.	40.5	279	*39*	10	4.1
*a*25	Sh.Sk. and L. 197 days; killed April.	46.0	328	*36*	10	3.5
	Average			*42.3*	13.5	

All animals included in this table are the offspring of the same female.
For explanation and abbreviations see explanation to Table I.

included in this table are the offspring of the same female. The average number of follicles in this brood is 42, the smallest number was 24, the largest 72. Among 31 animals there were seven possessing more than 50 follicles, and only three whose thyroids contained more than 60 follicles.

In Table IV. are compared two series which were raised under the same conditions, but were the offspring of two different females. There is a marked difference in the follicular number between the two series. The average numbers show a difference of 15.

In Table V. "lot *a*" of the series CCLXXI. is compared with "lot *b*" of the same series. Of the thyroids of the 8 animals of "lot *b*" seven (87.5 per cent.) contain more than 40 follicles, three (37.5 per cent.) more than 65 follicles; in "lot *a*" the thyroids of only two animals (8.3 per cent.) among 24, contain more than 65 follicles, 15 (62.5 per cent.) more than 40 follicles. The average number of follicles in "lot *a*" is 44, in "lot *b*" 54. Both lots are the offspring of the same female. But in "lot *a*" the larvæ were isolated, at an early stage, into individual finger-bowls; individual attention was given to each animal both in regard to food and water. The larvæ of "lot *b*" were left together in one large dish; the supply of oxygen was poor and the food scarce. Only at the end of the larval period they were placed under conditions similar to those of "lot *a*." The follicular number of the animals raised under favorable conditions is lower than the follicular number of those raised under poor conditions.

In Table VI. "lot *b*" of the preceding series is compared with series CCLXII. As mentioned before, "lot *b*" consisted of animals raised under unfavorable conditions of food and oxygen. Series CCLXII. was composed of larvæ of unknown parentage, collected in one of the ponds of the vicinity of Woods Hole and brought to the laboratory only shortly before metamorphosis These animals were extremely well nourished, of large size and had lived under optimum conditions. The difference in the follicular number between this series and "lot *b*" of the series CCLXXI. is still greater than the difference between the well nourished "lot *a*" of series CLXXI. and the "lot *b*." In series

TABLE IV.

THE PROGENY OF TWO DIFFERENT FEMALES OF *Ambystoma maculatum*, RAISED UNDER THE SAME CONDITIONS, ARE COMPARED REGARDING THE NUMBER OF FOLLICLES OF THE THYROID GLAND.

Series CCLXXIV.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Number of Follicles per cm². Area.	*Number of Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
1	Larval.	20.0	65	*52*	43						
2	Larval.	21.3	66	*28*	22						
*a*1	E.pr.d.	29.2	92	*49*	14						
*a*2	E.pr.d.	32.3	92	*29*	7	14	*41*	79	27.2	E.pr.sl.; yell.N.	2
						17	*56*	87	30.6	E.pr.d.; yell.N.; Op.shall.	3
*a*3	Pieces 1 day; E.pr.d.; yell.N.	33.7	96	*32*	11	11	*72*	88	31.1	E.pr.d.; Op.shall.	5
						14	*47*	91	32.4	Just shedding; Op. and G. open.	6
						17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
3	Sh.Sk. 0 days.	22.5	68	*48*	19						
*a*5	Sh.Sk. and L. 1 day.	—	106	*26*	10						
						16	*71*	92	31.7	Sh.Sk. and L. 2 days.	7
						21	*67*	95	28.6	Sh.Sk. and L. 3 days.	8
						17	*32*	87	28.8	Sh.Sk. and L. 4 days.	4
*a*4	Sh.Sk. and L. 5 days.	35.4	100	*47*	16						
	Average			*39*	18	16	*54*				

TABLE V.

TWO SERIES OF *Ambystoma maculatum*, COMPOSED OF THE OFFSPRING OF THE SAME FEMALE, BUT RAISED UNDER DIFFERENT CONDITIONS, ARE COMPARED WITH EACH OTHER REGARDING THE NUMBER OF FOLLICLES OF THE THYROID GLAND.

Series CCLXXI.*a*.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Number of Follicles per cm.² Area.	*Number of Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
1	E.pr.sl.	25.7	75	*42*	17						
2	E.pr.sl.	27.9	77	*51*	16						
4	E.pr.d.	31.1	77	*35*	15	14	*41*	79	27.2	E.pr.sl.; yell.N.	2
						17	*56*	87	30.6	E.pr.d.; yell.N.	3
5	E.pr.c.; yell.N.	35.9	88	*35*	13	11	*72*	88	31.1	E.pr.d.; yell.N.	5
6	E.pr.c.; Op. and G. closed.	35.6	89	*65*	12						
3	Just shedding; Op. and G. closed.	27.8	77	*30*	10	14	*47*	91	32.4	Just shedding; Op. and G. open.	6
12	Just Sh.Sk.; Op. and G. open.	—	91	*28*	9	17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
14	Sh.Sk. 0 days; Op. and G. open.	35.2	95	*24*	8						
7	Sh.Sk. 1 day; Op. and G. closed.	33.8	89	*50*	12						
9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11						
8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	16	*71*	92	31.7	Sh.Sk. and L. 2 days; Op. and G. closed.	7
13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6						
10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	21	*67*	95	28.6	Sh.Sk. and L. 3 days; Op. and G. closed.	8
11	Sh.Sk. and L. 4 days.	29.6	89	*43*	10	17	*32*	87	28.8	Sh.Sk. and L. 4 days.	4
15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7						
18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15						
19	Sh.Sk. and L. 11 days.	33.1	101	*36*	15						
20	Sh.Sk. and L. 12 days.	34.9	101	*50*	9						
16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12						
17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10						
21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6						
22	Sh.Sk. and L. 17 days.	35.3	102	*47*	14						
23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25						
	Average			*44*	12	16	*54*				

TABLE VI.

TWO SERIES OF *Ambystoma maculatum*, ONE RAISED UNDER OPTIMUM CONDITIONS (CCLXII.), THE OTHER ONE UNDER POOR CONDITIONS (CCLXXI.*b*), ARE COMPARED WITH EACH OTHER REGARDING THE NUMBER OF FOLLICLES OF THE THYROID GLAND.

Series CCLXII.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Number of Follicles per cm². Area.	*Number of Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
6	Larval; yell.N.	36.0	125	*10*	3	14	*41*	79	27.2	Larval; E.pr.sl.; yell.N.	2
4	Late larva; E.pr.	37.3	125	*35*	7	17	*56*	87	30.6	E.pr.d.; yell.N.; Op.shall.	3
10	Late larva; E.pr.	37.4	127	*30*	7	11	*72*	88	31.1	E.pr.d.; yell.N.; Op.shall.	5
8	Begins Shedd.Sk.; Op.shall.	37.3	126	*37*	8						
7	Begins Shedd.Sk.; Op.shall.	42.0	126	*25*	3						
						14	*47*	91	32.4	Just shedding; Op. and G. open.	6
2	Just Sh.Sk.; Op. clos.; G. open.	42.4	126	*31*	4	17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
5	Sh.Sk. ½ hour; Op. and G. open.	40.1	125	*5*	1						
9	Sh.Sk. 3 hours; Op. very shall.	37.0	126	*44*	8						
11	Sh.Sk. and L. 1 day; Op. and G. clos.	36.1	128	*30*	7						
12	Sh.Sk. and L. 1 day; Op. and G. clos.	32.4	128	*37*	10						
1	Sh.Sk. 2 days; L. 1 day; Op. and G. clos.	40.9	120	*39*	5	16	*71*	92	31.7	Sh.Sk. and L. 2 days; Op. and G. clos.	7
						21	*67*	95	28.6	Sh.Sk. and L. 3 days; Op. and G. clos.	8
14	Sh.Sk. 4 days; L. 3 days; Op. and G. clos.	41.4	130	*36*	4	17	*32*	87	28.8	Sh.Sk. and L. 4 days; Op. and G. clos.	4
	Average			*30*	6	16	*54*				

CCLXII. the average follicular number is only 30, while in "lot *b*" it is 54; there is a difference of 24 between the average follicular numbers of these two series. And it is again the series reared under better conditions, in which the follicular number is lower.

As far as we can trust these scanty figures it seems that the follicular number of the thyroid gland is influenced by hereditary and by environmental factors. Among the descendants of one particular female there may be more animals with conspicuously low or high numbers of follicles than among the descendants of other females. Animals growing up in a favorable environment have a lower number of follicles than those growing up in a less favorable environment.

We do not know what rôle the number of follicles might play in the function of the thyroid gland. Nevertheless it is known that certain pathological conditions are characterized by high or low numbers of follicles. It is of interest in this connection that the inclination towards a higher or lower number of follicles may be inherited.

Summary.

1. In the species *Ambystoma maculatum*, as contrasted to the species *A. opacum*, the number of follicles does not show, as a rule, the characteristic changes related to metamorphosis and consisting in the formation, by fusion of follicles, of a large, tube-like or bag-like follicle filled with colloid. Nevertheless a large, bag-like follicle making the largest part of the entire organ was found in one animal among 120.

2. In the species *A. maculatum* the average number of follicles is 39.7.

3. The number of follicles in each thyroid remains constant during the entire life of the individual.

4. The growth of the thyroid is effected entirely by an increase in the size of the follicles.

5. The primary follicles are simple spherical vesicles.

6. As the follicles grow in size the surface becomes markedly irregular. The large follicles possess diverticula of varying number, size and shape or may be composed of two or more equally large and diverticulated component follicles.

7. Separation of the bud-like diverticula from the mother follicles or cleavage of the larger follicles into smaller follicles does not occur.

8. The bud-like diverticula and composite follicles are the result of fusion between adjacent follicles.

9. The source for the continuous formation of new follicles are probably small masses of epithelial cells found in many thyroids.

10. Owing to the fusion of the primary follicles, which goes on at the same rate as the formation of new follicles, the number of follicles remains constant.

11. In adult, sex-mature animals primary follicles cease to develop; those present do not fuse with larger follicles, but themselves begin to enlarge in size.

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REACTIONS OF AN ELASMOBRANCH (*SQUALUS SUCKLII*) TO VARIATIONS IN THE SALINITY OF THE SURROUNDING MEDIUM.

J. P. QUIGLEY.

From the Pacific Biological Station, Nanaimo, B. C., and the Department of Physiology and Pharmacology, University of Alberta, Edmonton, Alberta.

A study of the changes which occur in fish when placed in a medium other than their natural habitat has attracted the attention of many investigators. Variations have been made both in the concentration and in the kinds of salts used and experiments of this nature have been conducted with teleosts and elasmobranchs. Our knowledge of the subject is, however, by no means complete, and a further investigation of certain phases was thought to be of value.

It is generally appreciated that elasmobranchs are usually less resistant to changes in salinity than are the teleosts. The former, therefore, make excellent subjects for an investigation of certain phases of the problem. *Squalus sucklii* was selected for the following investigation. This is a species of elasmobranch which has heretofore received little attention from physiologists.

In addition to observing the length of time this fish was able to live in certain solutions differing in amount and kind of salinity from its natural habitat an investigation was made of the changes in the gill movements, the heart beat, the amount of hemoglobin and the weight of the animal when taken from sea water and from the experimental solutions. A comparison was also made of the weight of the liver, spleen and pancreas taken from fish that had died in the various media.

The fish used in this research were taken on a set line from the Strait of Georgia near Departure Bay, Vancouver Island, and usually from a depth of 30 yards. The fish were handled very carefully but some injury always resulted from this method of capture. However, good specimens usually would live for a week when kept in cages fastened to a float in sea water despite

the fact that they were not fed during this time and that the salinity of this water was lower than that of their natural habitat. The fish were taken during the months of June, July and August of 1926 and a total of 219 were obtained suitable for this investigation. The fish were kept in a cage fastened to a float in sea water and were usually used within a few hours after being taken from the set line, and always within 15 hours of the time of capture.

For the purpose of comparison some physical characteristics are given of the solutions with which this investigation is concerned (Table I.).

METHODS.

In this investigation, the following media were used: (*a*) distilled water, (*b*) tap water, (*c*) tap water which had been given a pH of 8.4 by the addition of NaOH solution, (*d*) NaCl, in distilled water, (*e*) NaCl, $CaCl_2$ in distilled water, (*f*) NaCl, $CaCl_2$, KCl in distilled water, (*g*) NaCl, $CaCl_2$, KCl, $MgSO_4$ in distilled water, (*h*) sea water to which NaCl, $CaCl_2$, KCl, $MgSO_4$ had been added. In each case the quantity of the salt added was the amount required to give a concentration of the cation approximately the same as that found in sea water. When distilled water was used it was freshly made, cooled and then shaken with air. The water was obtained from a copper still but this is considered to be of negligible importance. The various solutions were frequently agitated during the course of the experiment and the fish were changed to a fresh medium of the same kind if the experiment continued for more than an hour.

Since it was desired to have conditions approximate as nearly as possible those which a fish would encounter if it were to swim naturally into the solution used, the fish were not "wiped." Before being placed in the solution they were allowed to hang head downward for one half minute, and then weighed. A slight amount of bleeding was produced by introducing the point of a scalpel having a thin blade into the caudal artery at the base of the caudal fin. Samples of blood were taken for the determination of the amount of hemoglobin and then the fish were placed in the desired solution.

TABLE I.

Medium.	pH.	Temperature Degrees C.	Density.	Total Solids gm. per Liter.	Oxygen Content cc. per Liter.	Grams per Liter.			
						NaCl	$CaCl_2$	KCl.	$MgSO_4$.
Water from which fish were captured	8.4	10.3 [1]	1.0218 [1]		4.41 [1]	27.37 [1]			
Water in cage where fish were kept	8.4	16–18	1.0184 [1]	28.72	5.43 [1]	22.17 [1]			
Distilled water	7.0	16–18	1.0000	0		0	0	0	0
Tap water	6.9	16–18	1.0003 [1]	0.087	5.32	Trace			
Tap water pH 8.4	8.4	16–18							
NaCl solution	7.0	16–18		27.0		27	0	0	0
NaCl, $CaCl_2$ solution	7.0	16–18		28.164		27	1.164	0	0
NaCl, $CaCl_2$, KCl solution	7.0	16–18		28.914		27	1.164	0.75	0
NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	7.0	16–18		35.414		27	1.164	0.75	6.5
						Added to sea water			
Sea water with added salts	7.0	16–18		64.134		27	1.164	0.75	6.5

[1] C. C. Lucas, personal communication.

It is well recognized that the effectiveness of a stimulus is partly dependent on the rate at which it is applied, therefore the sudden immersion of the fish in the solution should result in a maximum effect. Also, the injuries which the fish received incident to capture might alter slightly the results of an experiment.

After a variable length of time, the respiratory movements became slower and weaker. As the paralysis of the respiratory movements became more marked some struggling usually occurred and finally respiration completely failed. When respiration ceased, as was evidenced by cessation of movements of the spiracle, the time was noted, the fish was removed from the water, suspended head downward for one half minute as before, and reweighed. Blood was again taken for the determination of hemoglobin. The fish was placed on a tray, the heart exposed and if found beating, as was practically always the case, the time when the cardiac movements ceased was noted. The liver, spleen and pancreas were then exposed, freed from adjacent tissue and weighed.

Observations were made of the respiratory movements of all the fish used while in the various solutions but a series of thirteen fish were used in making a more complete study of the effect of tap water on the nature of the respiratory and cardiac movements. Freshly caught fish were weighed and then fastened to a wooden frame. The frame was submerged in a tank of sea water in such a manner that all of the fish, with the exception of the ventral surface directly over the heart, was covered by the water. A median incision was made in the exposed area, the tissue at the sides of the incision was retracted in such a manner that the heart was exposed, but the entrance of water to the wound was prevented. By means of a thread, the apex of the ventricle was attached to a heart lever arranged to write on a kymograph drum. A second heart lever was attached to one of the gill slits and arranged to record respiratory movements on the same drum. A record was made of the respiratory and cardiac movements of the fish in sea water. This water was then quickly drawn out of the tank and replaced by tap water and a second record was made. After the fish

had been in tap water for thirty minutes, a third record was made and then the water was replaced by fresh tap water. A fourth record was made after the fish had been in the tap water for fifty-five minutes. After the fish had been in the tap water for sixty minutes, it was removed from the water and killed by destruction of the central nervous system.

In determining the normal weight of the liver, spleen and pancreas 103 fish (34.9 per cent. females) of various sizes were used. These were fish that died in sea water either shortly before lifting the set line or soon after being placed in the cages. The fish were held head downward for one half minute to drain and then weighed. The abdominal cavity was opened by a median incision. The liver, spleen and pancreas were each dissected from the adjacent tissue and immediately weighed. From the figures so obtained, the weight of each organ per gram of fish was calculated.

Results and Discussion.

Toxicity of Media.—As a result of the studies of many investigators [for a review of this subject see Garrey (1, 2), Scott (3, 4), Macallum (5)] it has been shown that the osmotic pressure of the blood of a fish is rarely the same as that of its natural habitat. This is made possible by the relative impermeability of the integument, alimentary tract mucosa and of the gill membranes. The kidneys also play an important rôle in keeping the blood composition constant. However, when the fish is transferred to a medium having a different osmotic pressure the impermeability of the gill membranes is reduced and a passage of water and salts occurs which can not be entirely counter-balanced by the activity of the kidneys. This leads to a partial equalization of the osmotic pressures of the solutions separated by the gill membranes. In general, this equalization occurs more rapidly and more completely in elasmobranchs than in teleosts. Toxicity of a solution appears to some extent to be related to the degree to which it differs in osmotic pressure from that of the natural habitat of the fish. Therefore, teleosts will frequently survive changes in osmotic pressure of the external medium which would be fatal to elasmobranchs.

Distilled water has usually been found to be very toxic for fish, Ringer (6), Wells (7), but Garrey (2) found that fish can live in this medium for weeks and he quotes a similar observation made by Loeb. Garrey (2) has likewise corroborated the findings of Ringer (6) (8) that the toxicity of distilled water is reduced by the addition of a small amount of salts; for example, fresh water is less toxic than distilled water.

Many physiologists Loeb (9), Ringer (6), Garrey (2) have demonstrated with a number of species of marine and fresh water teleosts the mutual antagonism and progressive decrease in toxicity as Ca, then K, and finally Mg ions have been added in definite proportions to NaCl solutions. The usual explanation offered is that these ions exert their protective action by reducing the injurious effect which the individual ions have on the permeability of the gill membranes to salts and water.

In so far as I have been able to ascertain no previous investigation has been made on the effects of physiologically unbalanced solutions, with the possible exception of fresh water, on any elasmobranch or the effect of physiologically balanced solutions on *Squalus sucklii*.

In determining the effect of unbalanced, partially balanced and completely balanced solutions on dogfish it was found, that although the differences in the toxicity of the solutions used were not very great (Table II.), the results in general agreed rather well with those obtained by the other investigators. Using the time required for failure of the respiration as a guide to the toxicity, the solutions in order of their decreasing toxicity are Na > distilled water > Na, Ca, K, Mg, > Na, Ca, > Na, Ca, K. Since the solution containing the Na, Ca, K, and Mg ions was in substance an artificial sea water, it is difficult to explain its apparently high toxicity.

Using failure of the heart as a guide to the toxicity, the results are distilled water > Na > Na, Ca > Na, Ca, K, Mg > Na, Ca, K. The solution made by adding NaCl, $CaCl_2$, KCl, and $MgSO_4$ to sea water so that the cations would be present in approximately twice their normal concentration was a balanced solution. Therefore it is interesting to note that this solution as regards its effect on respiratory or cardiac movements was the

TABLE II.

Solution Used.	Number of Fish Used.[1]	Ave. Duration of Movements (in Minutes).		Ave. Change in Hemoglobin Gm. per 100 cc. Blood.	Ave. Percentage Change in Weight.
		Respiratory.	Cardiac.		
Distilled water	24	73	100	−0.613	+1.75
Tap water	23	113.2	144.7	−0.323	+3.66
Tap water pH 8.4	4	87.5	128	−0.487	+6.7
NaCl solution	10	71	137.7	−0.046	−1.09
NaCl, $CaCl_2$ solution	5	89	138	−0.035	−0.132
NaCl, $CaCl_2$, KCl solution	7	125	165	−0.0057	−2.379
NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	6	88.3	153.3	+0.108	−0.032
Sea water with added salts.	6	53.3	83.3	+0.345	+3.46

[1] Does not apply to determination of hemoglobin.

+ Indicates increase, − indicates decrease.

most toxic solution used. Experiments of Loeb and Wasteneys (10), Garrey (2) and Portier and Duval (11) also indicate that a balanced solution may be toxic when the concentration exceeds a certain limit.

The water referred to as tap water was a ground water caught in a small private reservoir and supplied to the Pacific Biological Station. It may be considered as similar to that which fish would encounter if they were to swim to a point above tide water in the streams around Departure Bay. This water had a very low salinity but it is quite possible that the cations Na, Ca, K and Mg were all present.

Tap water was found to require a longer time to produce cessation of respiration or cardiac failure than any of the experimental solutions with the exception of the Na, Ca, K solution, an observation which would strengthen the conclusion that toxicity is not a simple question of osmotic pressure. Since the dogfish continued to breath for an average of 113 minutes in tap water and remained active during most of this time, they probably could escape from a fresh water stream even if they were to swim into it above tide water level. As in the case of the salmon, Greene (12), the resistance of the dogfish to fresh water might be increased if it were to ascend the stream very gradually.

Scott (3), however, has shown that dogfish may be permanently injured by solutions whose osmotic pressure is markedly different from that of the normal habitat. This investigator has shown that when specimens of *Mustelus* were placed in either a hypotonic or hypertonic solution, the freezing point of the blood returned to normal either slowly or incompletely after being returned to sea water depending on the length of time the fish remained in the abnormal solution and the degree to which this solution differed from normal sea water. A similar conclusion may be drawn from the results obtained in this investigation for it was observed that after being left in distilled water or tap water until a disturbance of the respiration was observed, the condition of *Squalus sucklii* did not improve when transferred to sea water. The specimens of *Mustelus* used by Scott (3) in nearly every case died in less than 100 minutes when immersed in fresh water. Although it is likely that the tap water used in the experiments here described contained less salts than the fresh water used by Scott, *Squalus sucklii* (Table II.) continued to breath for an average of 113.2 minutes and cardiac failure occurred after 144.7 minutes.

Chidester (13) quotes the work of many investigators, from which it is to be concluded that fish are very sensitive to changes in the pH of the medium and, moreover, that the toxicity of a solution is less, the nearer it approaches the pH of the natural habitat. It was therefore considered of interest to determine whether the toxicity of tap water (pH 6.9) would be decreased by making the concentration of the hydrogen ion correspond with that of the normal sea water. For this purpose, NaOH was used rather than Na_2CO_3 so that the variation in the salinity would be slight. Contrary to expectations it was found (Table II.) that the water at pH 8.4 was more toxic than at 6.9. As will be shown later, the toxicity of abnormal media appears to be related to a depression of the respiratory center. It is therefore possible that the fish lived longer in the tap water pH 6.9 because this solution, being of an acid nature, was less depressant to the respiratory center than the tap water pH 8.4. It is also possible that the NaOH exerted a toxic action in some manner other than through an alteration of the pH. As will be shown later, the

fish in this solution underwent a marked gain in weight, a fact which would indicate that the solution was very injurious to the gill membranes.

It was impossible to demonstrate any relation between the size of dogfish and their resistance to changes in salinity. Similar reports have been made for teleosts by Young (14) but Bert (15) maintained that the larger fish are more resistant. The fact that most of the large females used by the author were pregnant may be used in explaining why these fish were not more resistant.

Bert (16) has reported that when fresh water fish were transferred to sea water, circulatory changes occurred in the blood vessels of the gills so that the appearance of these structures was decidedly changed. In my comparison of the gills of dogfish that had died in sea water with those of fish that had died in the experimental solutions no constant variation in the appearance was observed.

When the dogfish were placed in the experimental solutions their behavior was in all cases, much the same. They usually remained quiet, giving no indication that the medium was obnoxious to them, but they moved energetically when disturbed. A white slimy material collected in the water and on the bodies of the fish after they had been in the solution for some time, and in some cases opisthotonos, most marked in the region of the neck, was observed. Shortly before and after the respiratory movements ceased, struggling movements frequently occurred. The eyes were usually opaque by the time respiration ceased. The significance of the previously mentioned slime is not known, but it may indicate that the solutions had an action on the integument of the fish, perhaps stimulating secretion from the gland cells of the integument. The formation of slime in teleosts associated with changes in the environment has been reported by Young (14) and by Bert (16).

Many pregnant fish were used in the experiments but in no case did abortion occur as the result of introducing the fish into the experimental solutions. On opening the abdominal cavity of these fish, after cessation of the heart beat, the embryos were in all cases found to be dead. Several pregnant fish taken

directly from sea water were killed by destruction of the central nervous system. They were allowed to remain undisturbed for an hour or more and then the embryos were examined. In most cases they were found to be alive and active. The cause of death in embryos following the introduction of the mother into an abnormal medium is not known. Asphyxia and dilution of the blood probably play minor rôles in this phenomenon.

Gill Movements.—A gradual decrease in rate and amplitude of the respiratory movements followed the introduction of *Squalus sucklii* into a medium of abnormal salinity. Until the fish had been in the medium for about half the time they survived the change this decrease was usually slight and in some cases was absent, but during the latter half of the experiment the decrease in both rate and amplitude was more rapid.

For thirteen fish in sea water, following exposure of the heart, the respiratory rate varied between 18 and 73 but averaged 41.6 to the minute. The fish gave little or no evidence of injury as the result of the operation and the results obtained are believed to approximate rather closely those to be observed in the normal intact animal. One hour after placing the fish in tap water, the respiration of five had ceased and the respiratory rate of the remainder averaged 31.7 per minute.

Lyon (17) reports that the normal respiratory rate of the shark under experimental conditions varies between 18 and 30 per minute with an average rate of 23. A specimen of *Mustelus* examined by Scott (3) had a respiratory rate of 59 per minute just as the change from sea water to fresh water was being made. After sixty-seven minutes in fresh water this fish made only 8 very feeble respiratory movements per minute. A specimen of *Squalus acanthias* observed by the same investigator breathed while in sea water at the rate of 14 times per minute. Greene (12) states that the respiratory rate of the salmon varies between 60 and 120 per minute. Parker (18) records 35 to 40 respiratory movements in the normal specimen of *Mustelus* resting in sea water and 50 to 55 per minute in the fish swimming slowly.

Scott (3) noted that the respiratory and the heart rate in *Mustelus* were at times equal but they appeared to be little correlated. Lyon (17) found evidence to indicate that the heart

of the sand shark normally takes its rate from the respiration. In the present investigation it was observed that in *Squalus sucklii* the respiratory rate and cardiac rate seemed to be unrelated (except as considered later, when struggling or gill cleaning movements occurred). They were rarely equal and usually the respiratory rate was between two and three times as rapid as the heart rate, except shortly before the death of the animal when the respiratory rate was markedly decreased while the heart rate had changed but little.

Hyde (19, 20) has noted slightly convulsive movements of the gills occurring in the normal skate and suggests that these may be for the purpose of forcing more water through the gill apertures and thus removing foreign matter. Lyon (17) observed that such movements occur in the shark when any foreign body or solution enters the mouth or when a manipulation of the body occurs. Scott (3) found that such movements became very marked in *Mustelus* some time after being transferred to fresh water but decreased in intensity and frequency before the death of the fish. He intimates that the injurious action of the fresh water on the gill membranes may be the cause of the increase in intensity and frequency of these movements. The gill cleaning movements did not occur according to Scott (3) when similar experiments were carried out on *Squalus acanthias*.

Movements similar to those described were noted in *Squalus sucklii*. These appear to be a normal movement and were observed while the fish were in sea water or in one of the experimental solutions. A more detailed study was made of these movements as they occurred when the fish were in tap water. With *Squalus sucklii* in tap water the gill cleaning movement may begin during any phase of the respiratory movement and consists of a single or more rarely two or three vigorous movements of the gills; the normal rhythm and amplitude is then regained. There is no preliminary movement preceding the gill cleaning movement. In some fish the gill cleaning movements may not be observed for some time but usually they occur at fairly regular intervals of one or two minutes. With certain fish, the intervals between these movements decreased to reach a minimum about one half hour after immersion in tap water

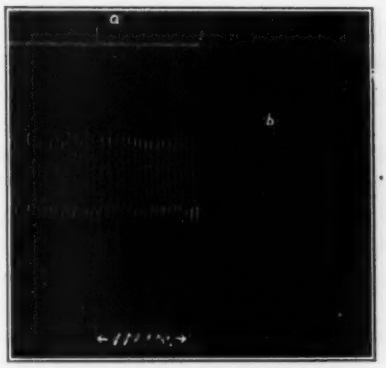
and then decreased in frequency until respiration ceased. This was not of constant occurrence, for cases were observed where the movements disappeared after the immersion of the fish in tap water. The amplitude of the gill cleaning movements was not increased by immersion of the fish in fresh water. In fact it could not be definitely stated that immersion of the fish in fresh water produced any constant variation in these movements.

During the ventricular diastole following the gill cleaning movement, or more rarely during the second ventricular diastole, the heart apparently loses tone and marked dilatation occurs, the heart loses a beat and then quickly resumes the normal tone and rate. Scott (3) observed a similar change in *Mustelus* and suggested that "the cardiac spasm" is an instance of reflex inhibition of the heart beat due to the cardiac inhibitory center being stimulated by impulses from the sensory nerves. Reflex cardio-inhibition does readily occur in fish, Lyon (17), Greene (12), but because of its rhythmic nature and for other reasons the explanation of Scott does not appear to apply to the movements which I observed in *Squalus sucklii*. I am, however, in accord with his conclusion that the respiratory convulsions do not produce the peculiar cardiac movements, but that the two processes have the same cause.

Distinction should be made between the movements described above and a somewhat similar movement observed in *Squalus sucklii*. This consisted of a spasm or series of struggling movements of the skeletal muscles but also involved the heart and gills. They appeared to occur during any part of the interval during which the fish were in the solutions and to originate in any external stimulus; occurring usually when the fish was touched or the water agitated. Several fish in a tank would remain quiet for a long interval, then a sudden movement of one individual would usually result in struggling movements among the others. These movements were more easily initiated after the fish had been in the solution for some time and especially just before and after respiration had ceased, that is, when the oxygen want might be expected to be greatest. The effect of these movements on the cardiac and respiratory movements was more pronounced but otherwise similar to those described as gill cleaning movements.

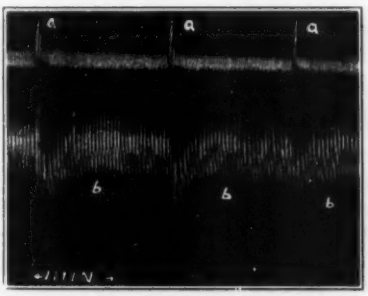
Although these movements would be likely to have a gill cleaning effect, this probably was not their primary object. They appeared to be the response given by the fish to a sensory

Simultaneous Records of Respiration (upper line) and Heart Beat (middle line).



Fish in sea water, (*a*) gill cleaning movement without a tonus wave in the record of the heart beat, (*b*) record on rapid drum.

After fish had been in tap water for 54 minutes, (*b*) record on rapid drum.



Record from fish after 6 minutes in tap water, (*a*) gill cleaning movements with (*b*) tonus waves.

stimulus after an increase in excitability had occurred. This increase in excitability may have been the result of the accumulation of CO_2 in the tissues due to the asphyxia which followed

the introduction of the fish into an abnormal medium. Bert (16) has described the agitation and irregular respiration occurring in fresh water fish transferred to sea water and Young (14) has observed that when fresh water teleosts were placed in a solution whose salinity differed from that of their natural habitat the fish were restless and easily excited before death. Wells (21) has noted a somewhat similar condition in fish dying in a solution containing a high concentration of carbon dioxide and a low concentration of oxygen. This apparently is the same condition as that described above for the dogfish. No other forms of irregularities in the respiration of the dogfish were noted. Cheyne-Stokes rhythm is at times observed in cases of anoxemia, I therefore expected that it might occur while the fish were in the experimental solutions but it was not observed. Lyon (17) has noted this type of respiration occurring in the shark shortly before death.

Cause of Death of Fish in Experimental Solutions.—Perhaps the most noticeable change which occurred in the dogfish when placed in the experimental solutions was a gradual but progressive slowing of the respiration terminating finally in a complete cessation of the respiratory movements. Since this change occurred with all of the solutions used, it does not appear to be the result of the presence or absence of any of the salts used nor of the pH of the solution. The rate at which failure of respiration becomes complete as shown in Table II. does vary with the salt or combination of salts used. This has previously been considered.

Many investigators studying the effect of variations in salinity have noted the injurious effect on the respiration and Lyon (17) has noted that the respiration ceases before the heart beat when sharks die in sea water, while Greene (12) from a study of the salmon concludes that the spawning act produces death through some more vulnerable channel than the heart and blood vessels. It may be a rule of more or less general application for fish that the respiratory apparatus succumbs more readily than the circulatory.

Assuming that the fish used in our experiments died from asphyxia, it is interesting to note that the solutions used contained

about as much oxygen at the beginning of the experiment as did the sea water from which the fish were captured. The solutions were agitated frequently and it is not likely that any marked decrease in the oxygen content occurred during the experiment. Moreover Powers (22) has noted that the oxygen content of the normal medium in which fresh water or marine fish live can be decreased to a low level (between 1.7–0.4 cc. per liter) before the fish will exhibit oxygen want.

That anoxemia was an important factor in the death of the fish placed in experimental solutions has been the conclusion of many investigators. Indeed, it has been shown by Backman (23) that if the sea water in which dogfish live, with $\Delta = -1.88°$ is diluted to $\Delta = -0.5°$, fifteen minutes after being placed in this solution, the tension of oxygen in the blood is diminished from 19.1 to 3.7 per cent. This anoxemia was believed to be a result of injury to the gill membranes and to the change in the water and salt content of the blood with the attendant change in size and the destruction of the blood corpuscles, thus decreasing the gas carrying power of the blood.

However, if fish were dying from asphyxia due to an injury of the gill membranes while the irritability of the respiratory center remained normal, hyperemia and dyspnoea might be expected. They did not occur in *Squalus sucklii*. The changes observed correspond more with those to be expected from a depression of the respiratory center.

As a result of this investigation and the contributions to this problem made by others, the author is inclined to explain the death of fish in media having an abnormal salinity to a progressive depression of the respiratory center. Although a change in the composition of the surrounding medium frequently alters the permeability of the limiting membranes of the body, the changes in the osmotic pressure of the blood and the injury to the gills, provided they occur, are considered to be only a contributary cause of death. The action of the abnormal medium on the respiratory center and perhaps on the motor nerves supplying the gills appears to be the primary cause of death. Changes in permeability of the limiting membranes are of importance in this regard insofar as they alter the activity of the structures

mentioned by (*a*) making it possible for salts to diffuse into or from the inner ear and brain cavity, (*b*) initiating a reflex over some cutaneous nervous structure (lateral line?), (*c*) altering the composition of the blood supplying these structures. The relative importance of these factors may, of course, be subject to variations. According to this concept, teleosts are more resistant to changes in salinity, either because the changes mentioned above occur less readily or because the fish are more resistant to the changes after they do occur. In support of mechanism (*a*) we may consider the suggestion of Loeb and Wasteneys (10) that toxic salts ($NaNO_3$, NaBr, KCl) in the external medium exert their action in this manner. Furthermore, it is to be expected that a disturbance of equilibrium might be concomitant with changes in the inner ear. This phenomenon has been observed shortly before death when fish were placed in solutions of abnormal salinity, Loeb and Wasteneys (10) and Young (14), in solutions of glycerine, Siedlecki (24), and in solutions of low oxygen and high carbon dioxide content, Wells (21). Maxwell (25) states that in most selachians the lymph of the vestibule is in free communication with the exterior sea water through the ductus endolymphaticus, and that it is reasonable to suppose that the density of the lymph would be practically equal to that of sea water. Since this condition is never encountered in teleosts it may be that we have here an important factor in the different susceptibility of these two groups of fish to variation in salinity.

Cardiac Movements.—Observations of the heart beat of *Squalus sucklii* were confined to fish in sea water, tap water and fish that were removed from one of the other experimental solutions following cessation of respiration. In general the heart is much more resistant to changes in the external media than is the respiration.

The heart rate of fish in sea water, taken shortly after exposure of the heart varied between 14 and 31 beats per minute with an average of 18.9.

In the experiments where records were made of the cardiac movements while the fish were in tap water very little change in the beat occurred. The fish were removed from the tap water

at the end of an hour, or earlier in those cases where the respiration ceased in less than an hour. No failure of the cardiac movements occurred during this interval. No irregularities nor loss of tone of the heart muscle occurred other than those changes associated with the gill cleaning and struggling movements. A slight decrease in the amplitude of the contractions did occur and the number of beats per minute at the end of the hour varied between 4 and 28 with an average of 14.6.

Scott (3) records a heart rate of 50 per minute in a pithed specimen of *Mustelus canis* immersed in sea water. After the fish had been in fresh water for an hour the amplitude of the beat was about the same as at the beginning but the rate was only one fourth as great. When the respiration failed the heart beat was forcible and strong but it failed rapidly after this.

This investigator also records that in a pithed specimen of *Squalus acanthias* placed in the same medium from which it was captured (brackish water) the rate was 16 per minute. One specimen was placed in fresh water and observed for five and one half hours. At the end of this time, the heart rate was 8 per minute.

Scott (3) observed Traube-Herring waves in blood pressure records made from pithed specimens of *Mustelus* and thought that they might be the result of the destruction of the spinal cord. They ceased when the animal was placed in fresh water. Greene (12) observed somewhat similar waves in the tracings made from the Chinook salmon but considered them to result from the rhythmical effects of the respiration on the blood pressure. Lyon (17) observed something similar to Traube-Herring waves in blood pressure tracings made from sharks in sea water.

Rhythmical variations in cardiac tone (tonus waves) were observed in *Squalus sucklii* but they were more prevalent while the fish were in tap water than with fish in sea water. They appeared to begin and sometimes to end with the gill cleaning movements. Respiratory waves were not observed in the heart records. (See figures, page 177.)

Changes in the Amount of Hemoglobin.—We determined the amount of hemoglobin in 44 fish before and after immersion in

the experimental solutions. Meischer's modification of Fleischl's hæmoglobinometer was employed in making these determinations. This apparatus was calibrated for human blood and since it was not checked against any other method, the absolute amount of hemoglobin recorded may not be correct. However, the same method was employed for all the determinations and the relationship between them is therefore of value.

The results obtained from normal fish varied between 0.68 and 5.14 grams of hemoglobin per 100 cc. of blood with an average value of 2.84. We were unable to demonstrate any definite relationship between sex or weight of fish and the amount of hemoglobin. In an examination of the blood of the skate, Harris (26) found that the percentage of HbO_2 in the blood of this animal varied between 3.1 per cent. and 6.2 per cent. He considers that the average value for the normal skate is between 3.5 per cent. and 3.8 per cent. By the use of Oliver's tintometer, v. Fleischl's hæmometer or the spectrocolorimetric method of Rollet he obtained results which were in close agreement.

The average changes in amount of hemoglobin shown in Table II. are in close agreement with the differences in osmotic pressure which the fish encountered when transferred to the abnormal media. They appear to result largely from the dilution or concentration of the blood which followed the change in osmotic pressure of the external medium. However, it has been observed by Hall, Grey and Lepkovsky (27) that in the menhaden asphyxia leads to an increase in the hemoglobin value. A similar change may have been operating in *Squalus sucklii*.

Changes in Weight.—When the impermeability of the gills is decreased as the result of introduction of a fish into a solution having a salinity to which it is unaccustomed, water and salts would enter or leave the blood and tissue of the fish depending on the direction of the difference in the osmotic pressure. The direction and extent of the exchange of water might be indicated by a study of the weights of fish before and after the change in the external media had been made. It has frequently been observed that teleosts gain in weight when in a medium having a lower osmotic pressure than normal and lose weight in a hypertonic solution, Greene (12), Portier and Duval (28).

However, it was found by Gueylard and Portier (29) that sticklebacks "unlike any other fish" gain in weight in hypertonic and lose weight in hypotonic solutions, while Sumner (30) and Scott (31) observed that fish frequently gained but later lost in weight in the same solution and Scott especially noted that the changes in weight varied greatly for individual fish.

Regarding the average changes in weight of *Squalus sucklii* recorded in Table II.: fish in distilled water, tap water and tap water with a pH of 8.4 made a gain in weight which may be ascribed to the greater osmotic pressure of the blood than of the environment. The fish in tap water gained 3.66 per cent., a result which agrees well with the observation of Scott (3) that the average gain in water in a number of the tissues of *Mustelus* after immersion in fresh water was 3.1 per cent. The fish in distilled water gained less than those in tap water a result which may be explained by the fact that the latter fish lived on an average of 40 minutes longer in the tap water than did the fish in distilled water. The fish in fresh water pH 8.4 made a much greater gain in weight than did the others although they remained in the medium only a few minutes longer than the fish in distilled water. The large gain may be taken as indicative of an extensive injury to the gill membranes by the alkaline solution. The osmotic pressure of the solution containing NaCl, $CaCl_2$, KCl and $MgSO_4$ would be about that of sea water. Practically no change in weight resulted in *Squalus* placed in this solution. The osmotic pressure of the solution of NaCl, $CaCl_2$, KCl; of NaCl, $CaCl_2$; and of NaCl is each progressively lower than sea water. A loss of weight was observed in fish placed in each of these solutions. Fish placed in sea water to which salts had been added gained in weight, although the osmotic pressure of this solution was greater than that of the blood.

In general, the length of time fish were able to live in a solution was not related to the change in the weight of the fish. These results as well as those of other investigators previously mentioned indicate that a study of the changes in weight gives results which are difficult to interpret. The amount of water and salts passing through the gill membranes and the amount of these

substances eliminated by the kidneys should largely determine the change in weight of a fish placed in an abnormal medium. Marked individual variations of these two factors may explain the peculiar results noted.

Variation in Weights of Liver, Spleen and Pancreas with the Weight of the Fish.—The weight per gram of fish of the liver, the spleen and the pancreas taken from fish that had died in sea water varies with the weight of the fish. Although individual fish frequently show considerable variations in the relative weights of these organs, when an average is taken of the results from a number of fish with approximately the same body weight, a rather definite relationship becomes evident (Table III.).

In fish taken dead from sea water the average weight of the liver per gram of fish rises rather rapidly from 0.066 for fish weighing between 300 and 999 grams to 0.115 for fish with weights between 3,000 and 3,999 grams. As larger fish are considered, the figure tends to remain comparatively constant; that for fish weighing between 6,000 and 6,999 grams being 0.117.

With the spleen, the greatest weight per gram of fish (0.0040) occurs in fish with a body weight between 300 and 999 grams. In larger fish, the figure falls in a rather regular manner until with fish having a total weight between 6,000 and 6,999 grams, the weight of the spleen per gram of fish is 0.0015.

The pancreases taken from fish that had died in sea water show a variation similar to that noted in the case of the spleens. With fish whose weight lay between 300 and 999 grams, the weight of the pancreas per gram of fish was 0.00286, and for fish weighing between 6,000 and 6,999 grams, the figure is 0.00142.

Pregnancy or sex did not appear to result in an alteration in the weight of the organs and therefore in this connection, these factors may be neglected. Insufficient data, however, is at hand to permit the formulation of an opinion as to whether the increased weight of the fish due to the presence of embryos was balanced by a compensatory decrease in the weight of the body of the fish or an increase in the weight of the organs. Since the weights recorded were obtained from fish captured only during

TABLE III.

AVERAGE WEIGHT OF ORGANS PER GRAM OF FISH.

Organ.	From Fish that Died in	Fish with Body Weight between (gm.)							
		300–999	1,000–1,499	1,500–1,999	2,000–2,999	3,000–3,999	4,000–4,999	5,000–5,999	6,000–6,999
Liver	Sea water	0.0660[44]	0.0769[9]	0.0940[6]	0.1104[22]	0.1156[8]	0.0984[5]	0.1235[6]	0.1176[4]
	Distilled H_2O	0.06324[16]			0.1213[2]		0.103[3]	0.0826[3]	
	Tap water	0.0602[9]	0.0756[7]	0.113[3]	0.114[1]	0.124[3]	0.0897[7]	0.0824[8]	
	Tap water pH 8.4				0.1114[3]	0.134[1]			
	NaCl solution	0.0740[4]	0.0664[1]		0.0904[1]	0.0468[1]	0.110[1]	0.0814[1]	
	NaCl, $CaCl_2$ solution			0.1027[2]	0.1246[1]	0.0984[1]			
	NaCl, $CaCl_2$, KCl solution	0.0704[5]			0.1140[1]				0.0894[1]
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.0658[2]	0.0556[1]	0.0880[1]	0.1155[2]				
	Sea water with added salts	0.0479[3]	0.0927[1]	0.0637[1]			0.0971[1]		
Spleen	Sea water	0.00407[44]	0.00416[9]	0.00345[6]	0.00275[22]	0.00271[8]	0.00203[5]	0.00167[6]	0.00153[4]
	Distilled H_2O	0.00475[16]	0.00418[2]		0.00300[2]		0.00289[3]	0.00189[3]	
	Tap water	0.00439[9]	0.00365[7]	0.00326[3]	0.00311[1]	0.00214[3]	0.00244[7]	0.00231[8]	
	Tap water pH 8.4	0.00312[1]			0.00325[2]	0.00261[1]			
	NaCl solution	0.00355[4]	0.00606[1]		0.00357[1]	0.00276[1]	0.00238[1]	0.00206[1]	
	NaCl, $CaCl_2$ solution			0.00349[2]	0.00254[1]	0.00189[1]			
	NaCl, $CaCl_2$, KCl solution	0.00390[5]			0.00232[1]				0.00163[1]
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.00439[2]	0.00324[1]	0.00346[1]	0.00296[2]				
	Sea water with added salts	0.00374[3]	0.00321[1]	0.00300[1]			0.00140[1]		

TABLE III. (*continued*).

Organ.	From Fish that Died in	Fish with Body Weight between (gm.)							
		300–999	1,000–1,499	1,500–1,999	2,000–2,999	3,000–3,999	4,000–4,999	5,000–5,999	6,000–6,999
Pancreas	Sea water	0.00286[44]	0.00253[9]	0.00224[6]	0.00199[22]	0.00167[8]	0.00170[3]	0.00139[6]	0.00142[4]
	Distilled H_2O	0.00337[16]	0.00248[2]		0.00211[2]		0.00173[2]	0.00145[2]	
	Tap water	0.00324[8]	0.00240[7]	0.00203[3]	0.00296[1]	0.00182[2]	0.00155[7]	0.00142[8]	
	Tap water pH 8.4	0.00239[1]			0.00266[2]	0.00258[1]			
	NaCl solution	0.00319[4]	0.00305[1]		0.00236[2]	0.00136[1]	0.00170[1]	0.00165[1]	
	NaCl, $CaCl_2$ solution			0.00266[2]	0.00198[1]	0.00150[1]			
	NaCl, $CaCl_2$, KCl solution	0.00299[5]			0.00171[1]				0.00093[1]
	NaCl, $CaCl_2$, KCl, $MgSO_4$ solution	0.00266[1]	0.00244[1]	0.00242[1]	0.00185[2]				
	Sea water with added salts	0.00243[3]	0.00227[1]	0.00242[1]			0.00140[1]		

A fish in tap water, weight between 7,000–7,999, weight per gram of fish; liver 0.103, spleen 0.00153, pancreas 0.00099.
Small figures indicate the number of fish used.

the summer months, nothing can be said regarding the occurrence of seasonal variations in the weights of the organs.

With fish that had died in the experimental solutions, the weight of the fish after cessation of respiration was used in calculating the weight per gram of fish for the organs examined. Although possible sources of error in the method of determining the weights of the organs per gram of fish may be pointed out, the methods used in determining these values for fish that had died in the experimental solutions are so nearly the same, that a comparison of the figures is certainly of value. In the case of fish dying in sea water and also in the case of fish dying in the experimental solutions the results would probably have been more uniform if a much larger series of fish had been available. In general the weights of organs taken from the two classes of fish were in close agreement. Weights of organs taken from fish that had died in distilled water usually did run *slightly* higher than those taken from fish that had died in sea water, while these latter weights in turn were usually higher than those obtained from fish that had died in sea water with added salts.

Gueylard (32) working with a fresh water teleost, the stickleback, found the normal weight of the liver to be 0.0435 grams per gram of fish but after a 24-hour sojourn in water containing 20 grams per liter of NaCl, the weight had decreased to 0.0420.

A marine fish such as *Squalus* might be expected to show an increase in the weight of the liver after being placed in distilled water and in tap water but the results obtained show that this is not the case.

It is the contention of Gueylard that a relationship exists between the weight of the spleen and the resistance of fish to changes in salinity. This investigator has found the weight of the spleen per gram of fish in four species of marine teleosts to average from 0.0005 to 0.00073, while the spleen of five species of fresh water teleosts ranges from 0.00065 to 0.0024. Since the figures for the dogfish which had died in sea water range from 0.00407 to 0.00153, this fish appears to have a weight of spleen corresponding to that of the fresh water teleosts.

In a study of the stickleback, a fish readily adaptable to changes in salinity, Gueylard (32) found the weight of the spleen

per gram of fish to be 0.00535 while the average figure for five less adaptable species of fresh water teleosts was 0.00065–0.0027. She has further stated that if the stickleback were placed in water of its natural habitat to which 20 grams of NaCl per liter has been added, the weight of the spleen per gram of fish at the end of 15 minutes was 0.00356 and at the end of two hours 0.00266. Somewhat similar changes were observed when eels were transferred from fresh water to sea water (33).

Introduction of a marine fish into fresh water might therefore, be expected to produce an increase in the weight of the spleen. The fact that this change was not observed with *Squalus sucklii* may be taken as an indication that a relationship exists between the inability of the dogfish to make a change in the weight of the spleen and its lack of adaptability to changes in salinity. A second hypothesis is that in *Squalus*, the change in weight of the spleen occurs slowly and has not progressed sufficiently before death takes place to make itself apparent. Neither of these explanations appears satisfactory. Gueylard (32) also reports that when sticklebacks were placed in water to which NaCl had been added, the spleen became flabby and the color which normally was brownish red had changed to reddish yellow. No changes in the color or consistency were observed by the author when the spleens taken from dogfish that had died in sea water were compared with those taken from the experimental solutions.

It is a pleasure to express my appreciation to the Biological Board of Canada by whom a portion of the expenses incurred in this work has been defrayed. I am also grateful to Dr. W. A. Clemens of the Pacific Biological Station for the many facilities extended, to Professor A. W. Downs and Professor William Rowan of the University of Alberta for helpful suggestions, and to Viola W. Quigley for assistance with the practical work.

SUMMARY.

1. An investigation has been made of the effect on *Squalus sucklii* of transference to the following media: (*a*) distilled water, (*b*) tap water, (*c*) tap water pH 8.4, (*d*) NaCl solution, (*e*) NaCl, $CaCl_2$ solution, (*f*) NaCl, $CaCl_2$, KCl solution, (*g*) NaCl, $CaCl_2$, KCl, $MgSO_4$ solution, (*h*) sea water to which NaCl, $CaCl_2$, KCl, $MgSO_4$ had been added.

2. The most toxic solution was sea water with added salts, the solution of NaCl, $CaCl_2$ and KCl was the least toxic.

3. Tap water had a relatively low toxicity, but tap water which had been given the same pH as sea water by the addition of NaOH was more toxic.

4. Cessation of respiration invariably occurred more readily than did failure of the heart.

5. It appears likely that respiratory failure is the cause of death of fish in abnormal media. This is believed to result from a depression of the respiratory center.

6. No variation which could be ascribed to pregnancy, size or sex was observed in the duration of respiration or heart beat, the change in amount of hemoglobin, weight of fish or of organs while in the experimental solutions.

7. Changes in the amount of hemoglobin closely paralleled the changes in the osmotic pressure of the external media.

8. An increase in weight usually but not invariably resulted from introduction of fish into hypotonic media.

9. It could not be shown that a change in the comparative weight of the liver, spleen or pancreas followed the introduction of the fish into an abnormal medium.

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THE LUMINOUS MATERIAL OF *MICROSCOLEX PHOSPHOREUS* DUG.

STANISLAW SKOWRON.

From the Biological Laboratory of the University, Cracow, Poland.

In my former paper ('26) I have described the luminescence of *Microscolex phosphoreus* and some of its chief characteristics, which seem to exclude the possibility of infection with luminous microörganisms in this species. The present note deals with the luminous material alone, and with its changes during the emission of light. The changes accompanying the light production have never been adequately studied in living forms, owing to technical difficulties or unsuitable material. The results of Hickling ('26) who investigated the luminous slime of a fish species, *Malacocephalus lævis*, differ in many points from my own observations on *Microscolex*.

The luminous slime of *Microscolex*,[1] usually discharged in small quantities and examined in a drop of tap water to keep it moist, consists of not very numerous small granules, which are situated within large, round cells, or set free into the surrounding fluid. Their round shape may be somewhat modified, owing to the close contact with other granules, and their diameter although

[1] *Microscolex phosphoreus*, of rather common occurrence in Southern Europe, has never been as yet reported from Poland. Through the courtesy of Mr. Z. Wasyliszyn I received some specimens found in one of the coal-mines situated near Cracow. Visiting this place I observed in one passage not used for over two years, about 230 m. below the surface, great quantities of *Microscolex* which in these special conditions of constant temperature and moisture had propagated very rapidly. Walking in darkness, hundreds of luminous points were seen, glowing brilliantly after every step. This species seems to need a higher temperature and that explains why it can live in mines, where it was occasionally introduced.

Contrary to the individuals I have examined in Naples and others which were recently sent to me from Naples, the coal-mine forms do not show the day-night rhythm in luminescence, a fact probably connected with the constant darkness in which they are living. The degree of irritability of the nervous system, regulating the ejaculation of the luminous slime, seems to be the same during the night and day time, owing to these exceptional conditions.

very variable does not exceed a few microns. In many cases the granules form compact aggregates within the cells and show sometimes Brownian movement which does not cease at higher temperature of 80° C. and in 90 per cent. alcohol. They are of greenish color, strongly refract the light and do not show any optical activity. Under high magnification one can easily see that every granule is composed of two substances, one of which forms the central part, while the second lies around the periphery, an observation which appears to be similar to those of Förster ('12) on *Pholas* and of Hickling on *Malacocephalus*.

The granules seem to be highly resistant to cytolytic reagents (chloroform, saponin, xylol, etc.) and do not change much after a long time. In hydrochloric acid, however, they swell to some extent and the central part of each granule dissolves at once, while the outer layer can be preserved for a few minutes without any visible changes. The next step is the slow dissolution of the latter. The granules are also quickly destroyed by pepsin solution at a certain pH and about 40° C. but the changes are markedly different from those produced by hydrochloric acid.

The picture of the slime if examined immediately after ejaculation and without any addition of water is very different. Besides the cells and granules mentioned above, there are great masses of large cells filled completely with granules, the other cells being already broken and the granules set free. If now a drop of water be put near the edge of the cover-glass, it is possible to observe how in the moment of contact with diffusing water the bright flash of light is produced and the granules dissolve instantaneously leaving sometimes but a very transparent sheath similar to the shadows of red blood corpuscles in hypotonic solution. During this process many cells are breaking up, but some of them remain intact and the water passing through the permeable cell membrane dissolves the granules situated in the cell. In this case one can see afterwards a fine network within the cell which represents the rest of the dissolved granules. As far as I was able to observe, the granules dissolving in water look quite as the formerly described ones, except that their size is usually greater and it seems to me that they are but fully developed stages of the same kind of structures. This is further corroborated by the fact that the

undeveloped granules are never as numerous in the single cell as the others.

It is necessary to mention that every cell contains only undeveloped or developed granules alone, and in the slime one can always find both kinds of these cells, sometimes the cells with undeveloped granules being more numerous than usual but never as abundant as the others. Studying under the cover-glass the dissolution of the granules and the luminescence which lasts as long as granules are present, one must regard the dissolving granules as the real luminous material, a conclusion which disproves entirely the possibility of infection with luminous bacteria.

In order to find out the necessary conditions for luminescence I have first tried the effect of osmotic pressure of the surrounding medium, which, as pointed out by Hickling, plays a chief rôle in the diffusion of luminous substances from the granules of *Malacocephalus*. The experiments were, however, without result, as the luminous slime glowed in saturated KCl solution almost as bright as in distilled or tap water, the granules dissolving rapidly. Many similar observations showed that the osmotic pressure and different ions have no effect whatever on dissolution and luminescence of the luminous material. Glucose solutions gave different results. In higher concentrations both glucose and glycerin suppressed luminescence of the slime, which returned, however, upon addition of water. It seems that the amount of water regulates the dissolution of the granules and thereby the luminescence. Filter paper saturated with luminous slime ceases to glow in alcohol absolute and the granules remain intact, but the luminescence returns if some water is added. Taking for granted the importance of water, it will be easy to explain why the luminous slime, when ejaculated, glows longer in a moist atmosphere than in pure water, its quantity being the same in both cases. Larger drops of slime can be luminescent even in pure water for a few minutes because they form viscous clusters through which the water diffuses slowly.

I am not quite certain what prevents the luminescence of the granules inside the body of the worm. The amount of oxygen is ruled out of the question because the slime can be made luminous in water where the oxygen is in such small quantity that colorless

reduced methylene blue does not turn blue. The amount of oxygen necessary for luminescence must be very small indeed, contrary to the observations on *Malacocephalus*, and without any doubt smaller than its supply in the cœlomic fluid of the living animal. Greater quantities are necessary for the ejaculation of the slime only, which function however is under control of the nervous system. The amount of water in the secreted fluid is also sufficient because the slime gives off the light for some time even in dryness, not as long however as to use up all the luminous material, and here lies the explanation why the dried worms or slime are still luminous upon moistening. The process of breaking up the cells always occurs when the luminescence proceeds and the amount of water is not too abundant, because in this case the dissolution of the granules may take place within the cell membrane. It seems to me that the changes occurring normally during or after the ejaculation of the slime in the cells themselves, probably in their cortical layer, lead to the contact of granules with surrounding water and this causes their luminescence.

As I have already pointed out, the highly concentrated glucose solution, alcohol abs., and also pure ether and chloroform stop the luminescence but the process is reversible if water is added and the action of these reagents is not too prolonged. Quite different is the effect of $HgCl_2$, concentrated solution or diluted to one half with water. The power of luminescence is in this case irreversibly destroyed. The same effect can be obtained with alcohol abs. acting for over half an hour.

The granules are luminescent at pH from 3 to 11. More acid or alkaline solution extinguishes the luminescence irreversibly. It was impossible to show quantitatively the duration of luminescence in relation to different temperatures, owing to the difficulty in getting exactly the same quantities of slime in all cases. Nevertheless it was found that the light is fainter and of shorter duration both in higher (40–50° C.) and lower (−5–10° C.) temperatures.

Discussion.

It seems more than probable that the two substances which compose the luminous granules are both necessary for luminescence. We may suppose that these two substances represent

luciferin and luciferase separated from each other by a film as suggested by E. N. Harvey, while discussing the luminescence in other animals. When the cell breaks up and water is absorbed by the substances of the granules, the film may be destroyed and the fluid mixture glows till the whole material is used. It is possible that this destruction can be accelerated by the small addition of ether or chloroform to the water because of the effect of these solutions upon the intensity of luminescence, as I have formerly described. If the water is present in a very small quantity, the structure of many granules is not changed and the power of luminescence can be retained. The same result can be obtained when strong alcohol is added. Other substances, *e.g.*, $HgCl_2$, change chemically or physically the composition of the luminous material and these changes are irreversible. Osmotic pressure of the surrounding fluid does not play any rôle, contrary to the observations of Hickling on *Malacocephalus* who did not see the dissolution of the granules because he thinks that "in the artificial conditions of experiment the granules never become exhausted, since conditions automatically become unsuitable long before exhaustion occurs" owing to the too low pH for luminescence. In *Microscolex* the material was suitable enough to observe the dissolution of the granules and further investigations may show if this process is of more universal occurrence, because, as far as I know, it has only been mentioned by E. N. Harvey in jellyfishes by the action of saponin, sodium glycocholate and of fresh water. Besides, it is possible that different types of changes in luminous material may be met with, of which *Microscolex* and *Malacocephalus* are but two examples.

I am greatly indebted to Professor E. N. Harvey for criticizing my results.

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FUNCTIONLESS MALES IN TWO SPECIES OF *NEUROTERUS*.[1]

J. T. PATTERSON.

In connection with a proposed cytological investigation of the Cynipidæ, I have found it necessary to make a rather extensive collection and study of the different species present in the Austin area. Many new species and varieties have been discovered. These have been turned over to Dr. A. C. Kinsey for identification and description. Dr. Kinsey has already included a number of the Austin species in his important publications on the Cynipidæ, and others will be dealt with in future papers. I wish to take this opportunity to express my appreciation of his fine coöperation in this work.

During the course of this study many interesting facts concerning the biology of the Cynipidæ have been brought to light. Not the least among these is the discovery of functionless males in the (so-called) agamic generation of two species of *Neuroterus*. The species concerned are *N. contortus* (Weld) and *N. rileyi* (Bassett). Dr. Kinsey has recently redescribed these species and their varieties.

Neuroterus contortus has two generations a year, one called "*N. contortus* agamic form *contortus*," the other "*N. contortus* bisexual form *principalis*" (Kinsey, '23, pp. 91–92). Here at Austin this species is restricted to a single locality. Indeed, it is found in small clumps of sprouts around the base of two trees of *Quercus breviloba*. The galls of the agamic generation are found on the roots of the young shoots, just below the surface of the soil. The adults of this generation emerge during the last week of January and the first two weeks of February (1923). The female cynipid oviposits immediately in the tissues of the newly formed sprouts, just above the old galls, and usually above the level of the surface of the ground. By the middle of February

[1] From the Zoölogical Laboratory of the University of Texas. Contribution No. 209.

the new galls begin to appear and are fully formed by the middle of March. The adults of the bisexual generation emerge during the first two weeks in April (April 3–15, 1922). New galls of the next agamic generation were first recognized on May 19, 1922.

There is an interesting difference in the behavior of the females of the alternating generations. Those of the agamic generation react positively to light, while those of the bisexual generation react negatively. This was determined by tests in the laboratory, as well as by observations in the field. This difference in behavior may account for the relative positions (the agamic gall on the root, the bisexual on the stem) of the galls of the two generations.

I have bred out from the galls of the bisexual generation a total of 576 females and 1,076 males. This apparent disproportion of males to females is not due to any real difference in sex ratios, but is to be accounted for on an entirely different basis. I have found in this, and in certain other species of Cynipidæ, that all of the adults emerging from a single, isolated gall are of the same sex. This means that the agamic females must be of two kinds, one female- and the other male-egg-producing. If one collected galls a majority of which happened to contain adult wasps of one sex, this would give a larger number of individuals of that sex in the totals.

From galls of the agamic generation I bred out 231 females and 10 males. It is this interesting fact that forms the basis for the present note. I suspected from the first that these males might be functionless, and consequently decided to test out their sex behavior. On February 5, 1923, I bred out 62 females and 6 males from one lot of galls. As soon as they emerged the males were isolated and kept in a vial for several hours. Females were then placed with these males, and the culture kept under observation for an hour. During this period no matings took place. The females did not display any sex reactions, but some of the males did exhibit certain of the "courtship" reactions, such as approaching the female and vibrating the elevated wings. A single male made a very weak effort to mate with a female, but copulation did not occur. The experiment was repeated in the afternoon, but no attempts to mate were observed.

In order further to test out the behavior of these wasps, I collected a number of galls of the agamic generation and placed them in the ice box, on February 25, 1923. These were left there until the bisexual generation emerged in April. On April 14 the galls were taken from the ice box and kept at room temperature, and on the following day four agamic females came out. These were then tested with males of the bisexual generation. Before making the tests, however, the behavior of the males of the bisexual generation toward the females of that generation was studied. The normal sex reaction is as follows: As soon as virgin females are introduced into the dish containing the males, the latter become exceedingly active, and race rapidly about the dish. The male then approaches to within two or three millimeters of the female, stops, raises the wings above the body, and then rapidly vibrates the elevated wings. If the female responds, mating follows immediately. During this courtship on the part of the male, the female assumes a passive attitude, by becoming quiet, and then lowering the antennæ until the tips rest on the bottom of the dish. She remains perfectly motionless during the process of mating.

The four agamic females were introduced to a group of newly emerged males of the bisexual generation. The behavior of these males in the presence of the agamic females is identical with that described above. They show the normal sex reactions. On the other hand, these females do not give the slightest evidence of response. In fact, they make every effort to escape from the aggressive males. During the course of an hour about fifty attempts were made by the males to mate with these females, but there was not a single successful copulation. It would have been interesting to study the behavior of these males in the presence of the sexually active females of the bisexual generation, but unfortunately no males emerged from the refrigerated material.

It is evident from these observations that the agamic females have entirely lost the mating instinct. It is also clear that the aberrant males have nearly lost this same instinct and, so far as reproduction is concerned, are functionless.

The second species in which similar functionless males have

been found is *Neuroterus rileyi* variety *mutatus* (Kinsey, 1923, p. 118). Only the agamic generation of this variety is known. It produces an enlargement of the stems of *Quercus Muhlenbergii*. From April 4 to 11, 1922, I reared 94 females and 3 males from three galls. The sex behavior of these males is similar to but weaker than that of the functionless males of *contortus*.

The presence of a few aberrant males in the agamic generation of species showing the typical alternation of generations is of considerable interest, because it indicates that such males represent a remnant of a more primitive, bisexual condition in the generation which has now become agamic. As Kinsey points out, these observations are of considerable importance in connection with our understanding of the development of agamy and heterogeny in the Cynipidæ. I have no doubt that careful observations will reveal the presence of similar males in the agamic generation of other species. In most species, however, such males are evidently not present.

Another point of interest is the one concerning the origin of these males. Our cytological studies indicate that males of all species of the Cynipidæ are haploid, while the females are diploid. Males arise from unfertilized eggs that have undergone chromatin reduction. The females of the bisexual generation come from eggs which have failed to reduce the number of chromosomes, while those of the agamic generation develop from fertilized eggs. I have accumulated evidence which offers a possible explanation of the fact that the bisexual generation produces an agamic generation of females. In several species examined, the sexual females usually do not oviposit until after fertilization takes place. Eggs laid by these females should be fertilized and consequently would produce females. The few functionless males appearing in this generation might arise in one of two ways: either certain unimpregnated females occasionally lay eggs that develop into males or else an inseminated female lays a few unfertilized eggs, after the manner of the queen bee. Such eggs would produce males.

The suggestion that certain males are functionless in the Cynipidæ is not entirely new. Thus Adler ('81) has pointed out that in the genus "Rhodites" the few males found in certain species must be superfluous, but so far as I am aware the two cases

cited above are the first to be recorded for the agamic generation of heterogenous species.

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